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Formulation of Rifampicin Suspension

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Abstract

Rifampicin is the drug of choice in treatment of tuberculosis. Also, it is effective in treatment of various bacterial infections. This study was carried out to prepare a stable suspension for rifampicin through preparation of different formulas of rifampicin aqueous suspension either as ready to use or as granular powder to be reconstituted. The selected formula (A) was evaluated and compared with commercial brand of rifampicin (Rifactine®) as a reference through measuring their dissolution rates and other physical properties. The results indicated that the selected formula had better dissolution rate compared with the reference suspension and the rheogram showed that the selected formula was less viscous than the reference one. Also, it was found that the granular rifampicin was more stable than the ready to use suspension, since the expiration date of granular rifampicin was 2.6 years, while the expiration date of ready to use suspension was 1.8 years.

Keywords: Rifampicin, Suspending agent, Powder for reconstitution, Aqueous suspension

Introduction

An oral pharmaceutical suspension has long been one of the most favorable dosage forms for pediatric patients or patients unable to tolerate solid dosage forms (1).

There are many physical and chemical considerations in the preparation and development of a suspension to satisfy its pharmaceutical requirements. Some suspending agents are generally added to the dispersion medium in order that their structures help to maintain uniform dispersibility (2) or to prevent caking of the drug particles during shelf-life (3).

Rifampicin is bactericidal agent against wide range of microorganism (4). It is one of the very slightly soluble drugs, thus is suitable for suspension dosage form. But rifampicin is poorly wetted with water due to its hydrophobic nature.

El-bary et al. studied the wettability of rifampicin powder using different concentrations of various surfactants and polyhydroxy compounds. The wettability of rifampicin was found to be directly proportional to surfactant HLB and concentration (5). In this study, rifampicin is formulated as an aqueous suspension either as ready to use or as dry powder and the selected formula is compared with the reference suspension.

Experimental

Materials and Equipments

Rifampicin powder, Polysorbate 80, Raspberry flavor, Xanthan gum, Guar gum, Methylparaben, Propylparaben (Supplied by Samara Drug Industries (SDI)).

Received 26-11-2005
Accepted 24-5-2006
Rifactine® suspension (Supplied by Medical Union Pharmaceutical, Egypt)  
Sodium saccharin, Sorbitol 70%, Sodium citrate (Supplied by Ibn-seina Drug Research Center, Baghdad).  
Citric acid monohydrate (Al-Rahma Pharmaceutical Co, Jordan).  
Disodium edetate, Sodium metabisulphite, Hydrochloric acid, colloidal Silicone dioxide (Aerosil), Methylcellulose, Sodium carboxymethylcellulose (BDH chemicals Ltd, Poole, England).  
Spectrophotometer (Pye-Unicom-sp-8-100 model 292MK, England).  
Dissolution apparatus (Erewka G.M.B.H type DT6, W.Germany).  
pH meter (Orchidis laboratories, France).  
Viscometer (Cole-parmer, rotational viscometer U.S.A).  
Ovens (Memmert 854 Schwabach, W.Germany).  

**Method of preparation**  

**Formulation of Rifampicin Suspension:**  
Several formulas of rifampicin aqueous suspension were prepared, either as ready-to-use aqueous suspension or dry powder for reconstitution.  

**Ready-to-use aqueous Rifampicin Suspension**  
Different formulas of rifampicin suspension were prepared using different suspending agents as shows in table (1), each formula was prepared as follows:  
Rifampicin, methylparaben and propylparaben were levigated in a mortar with the prepared dispersion of the suspending agent. The mixture was triturated with a pestle until a smooth paste was formed. With continuous triturating, the paste was diluted with the remaining amount of the dispersion of suspending agent then transferred to graduated cylinder. The required amount of sodium saccharin and disodium edetate or sodium metabisulphite were dissolved in a small portion of distilled water and added to the graduated cylinder. Finally sorbitol, glycerol and raspberry flavor were added followed by adding sufficient distilled water to make up to volume. The suspension was shaken thoroughly and the pH was adjusted to 5 with few drops of 5M sodium citrate.

<table>
<thead>
<tr>
<th>Material</th>
<th>Formula</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>A 2</td>
</tr>
<tr>
<td>Rifampicin</td>
<td></td>
</tr>
<tr>
<td>Agar</td>
<td></td>
</tr>
<tr>
<td>Methylcelulose</td>
<td></td>
</tr>
<tr>
<td>Xanthan gum</td>
<td>x</td>
</tr>
<tr>
<td>SCMC</td>
<td>x</td>
</tr>
<tr>
<td>Polysorbate 80</td>
<td>x</td>
</tr>
<tr>
<td>Disodium edetate</td>
<td>0.1</td>
</tr>
<tr>
<td>Sodium metabisulphite</td>
<td></td>
</tr>
<tr>
<td>Glycerol</td>
<td>5</td>
</tr>
<tr>
<td>Sorbitol 70 %</td>
<td>5</td>
</tr>
<tr>
<td>Sodium saccharin</td>
<td></td>
</tr>
<tr>
<td>Methylparaben</td>
<td></td>
</tr>
<tr>
<td>Propylparaben</td>
<td></td>
</tr>
<tr>
<td>Raspberry flavor</td>
<td></td>
</tr>
<tr>
<td>Final volume</td>
<td></td>
</tr>
</tbody>
</table>
Powder for reconstitution

Different suspending agents were used to prepare rifampicin suspensions as powder form ready for reconstitution as shown in table (2). Each was prepared by triturating rifampicin powder with the selected components. The powder mixture was passed through a sieve (150 μm) before being transferred to amber glass bottles. There was an exception for formula I, in which a powder blend was moistened with (0.5% polyvinylpyrrolidone in alcohol). The damp mass was then passed through the sieve (1000 μm) and the granules were dried at 37 °C. The dried granules were resieved through the same sieve before being transferred to amber glass bottles. The ease of reconstitution and stability were evaluated to select the proper formula, which will be subjected to further study.

Table (2): Different Formulas of Rifampicin Powder to be Reconstituted as Suspension (% W/V)

<table>
<thead>
<tr>
<th>Material</th>
<th>Formula</th>
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<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>2</td>
</tr>
<tr>
<td>Guar gum</td>
<td></td>
</tr>
<tr>
<td>SCMC</td>
<td>x</td>
</tr>
<tr>
<td>Aerosil</td>
<td></td>
</tr>
<tr>
<td>polysorbate 80</td>
<td>x</td>
</tr>
<tr>
<td>Sucrose</td>
<td></td>
</tr>
<tr>
<td>Sodium saccharin</td>
<td></td>
</tr>
<tr>
<td>Methylparaben</td>
<td></td>
</tr>
<tr>
<td>Propylparaben</td>
<td></td>
</tr>
<tr>
<td>Disodium edetate</td>
<td></td>
</tr>
<tr>
<td>Sodium citrate</td>
<td></td>
</tr>
<tr>
<td>Citric acid</td>
<td></td>
</tr>
<tr>
<td>Raspberry flavor</td>
<td></td>
</tr>
</tbody>
</table>

Comparative Studies of The Selected Formula with Rifactine® Suspension:

The selected formulas (A and I) were compared with the reference Rifactine® utilizing the following parameters:

Dissolution Profile

The dissolution rate of rifampicin suspensions was studied using USP dissolution apparatus. The dissolution medium was 0.1N HCl (900 ml), 5 ml sample of suspension was added. Then a sample of dissolution medium was withdrawn at different time intervals (2, 5, 10, 15, 30 and 45 minutes) through a pipette fitted with a filter paper. Fresh dissolution medium was added to the jar each time to replace withdrawn samples. Each sample was suitably diluted and assayed spectrophotometrically at 475 nm for rifampicin content.

Measurement of Rheogram

Rheograms were obtained at 37 °C using Cole-parmer rotational viscometer.

Sedimentation Volume Measurement

Fifty ml of each suspension was diluted with distilled water to a volume of 100 ml in a stoppered graduated cylinder. The suspensions were shaken vigorously to ensure uniformity, then left undisturbed. The sedimentation volume was measured every 4 hours for period of 48 hours.

Resuspendability of Suspension

The test consisted of manually shaking the cylinder after the sedimentation experiment was completed. Based on the effort required to convert the sedimented system to a homogenous suspension, the prepared product was rated as: resuspendable, resuspendable with difficulty or not resuspendable.

Stability Study:

The accelerated stability study was done in order to determine the expiration date of formula A and I by placing the samples of both formulas in ovens at 35 °C, 45 °C and 55 °C for 120 days.

Samples were taken and assayed for drug content at suitable time intervals (0, 15, 30, 60, 90, 120 days) using UV spectrophotometric method at 475 nm.

Result and Discussion

The nonionic surfactant (polysorbate 80) was incorporated in the formulation of rifampicin suspension as a wetting agent to increase dissolution rate of the drug. In addition, xanthan gum was also used as a suspending agent because of its excellent suspending properties and also as an effective flocculating agent at relatively low concentration. An increase in the concentration of xanthan gum (as in formula...
B) gave no substantial change in flocculation behavior and a viscous suspension was obtained which pour with difficulty.

On the other hand, sodium carboxymethylcellulose (SCMC) was used as in formula D. It gave good dissolution properties and it produced sediment layer that was easily redispersed by shaking.

Furthermore, a combination of xanthan gum and SCMC resulted in too viscous suspension, which poured with difficulty. Methylcellulose utilized in formula E produced a suspension with low dissolution rate as shown in figure (1).

Figure (1) : Dissolution rate profile of rifampicin suspension (formula E) in 0.1 N HCl at 37ºC.

Finally, agar was utilized as thickening agent and to control flocculation. Good results were achieved with formula F but hard cake was formed with formula G.

Formula A gave the most optimum physical stability and remarkable release profile, therefore it was chosen for extensive study and to be compared with reference suspension.

On the other hand, rifampicin suspension (as dry powder) when prepared using guar gum as a single suspending agent (formula II) resulted in a suspension that showed low sedimentation volume (0.2) but was easily redispersed. The addition of aerosil to formula III and IV resulted in easily redispersed suspensions with high sediment volume (0.8 and 0.9 for III and IV, respectively). Being finely divided, aerosil aggregates to form three dimensional network together with its ability to absorb large amount of water, hence it prevents caking.

In addition sodium carboxymethylcellulose was used as a suspending agent in combination with polysorbate 80 to enhance the dissolution (formula I). This formula was prepared as granules using alcoholic PVP solution as a granulating agent. The granules were found to be free flowing and not bulky. Also, rifampicin granules were found to be good in appearance and their particles were uniform in size.

Formula I was chosen since it gave good stability although it produced sediment layer with easily redispersability by shaking.

Figure (2) shows the dissolution rate of rifampicin suspension for formulas A and I compared with the reference rifactine suspension. The results showed that rifampicin released from formula A was higher than that from others. Formula I after reconstitution showed the lowest dissolution rate and this may be due to granulation process, since PVP was used as granulating agent which is water soluble binder and has good swelling and hydration capacity. These properties result in high viscous region surrounding the drug particle.

Figure (2) : Dissolution rate profile of rifampicin suspension (formulas A and I) and rifactine in 0.1 N HCl at 37ºC.

Rheograms of the products are represented in figure (3). The graph showed that the viscosity of rifampicin suspensions was shear rate dependent and increased in the following order:

Formula A < Rifactine < Formula I

The results illustrated that the prepared formulas (A and I) exhibited pseudoplastic flow properties due to the suspending agents used, which were xanthan gum and sodium carboxymethylcellulose.
Tables (3 and 4) show the sedimentation volume and resuspendability after settling of rifampicin suspensions, respectively. The data indicated that formula A, which was prepared with xanthan gum, had sedimentation volume equal to 2. This result was attributed to the network of flocs formed in the suspension, which was so loose and fluffy that can be extended throughout extravehicle. The same result was reported by Jawad (10).

<table>
<thead>
<tr>
<th>Products</th>
<th>Sedimentation volume</th>
<th>Resuspendability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifactine</td>
<td>0.9</td>
<td>easily resuspended</td>
</tr>
<tr>
<td>Formula A</td>
<td>2</td>
<td>No sedimentation</td>
</tr>
<tr>
<td>Formula I</td>
<td>0.4</td>
<td>easily resuspended</td>
</tr>
<tr>
<td>Rifactine</td>
<td>0.9</td>
<td>easily resuspended</td>
</tr>
</tbody>
</table>

In the stability study of rifampicin suspension and granular rifampicin figures (4 and 5) showed that the degradation of rifampicin for formulas A and I respectively, which follows first order kinetics since straight lines were obtained by plotting the logarithm of percent remaining of rifampicin versus time.
The degradation rate constants (K) at different temperatures were calculated from the slopes of the straight lines and they were listed in Table (5).

**Table (5) Degradation Rate Constants of Rifampicin Suspensions Ready-to-Use (Formula A) and Granular Suspension (Formula I).**

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>K x 10⁻³ (day⁻¹) Formula A</th>
<th>K x 10⁻³ (day⁻¹) Formula I</th>
</tr>
</thead>
<tbody>
<tr>
<td>55</td>
<td>1.206</td>
<td>0.822</td>
</tr>
<tr>
<td>45</td>
<td>0.619</td>
<td>0.429</td>
</tr>
<tr>
<td>35</td>
<td>0.318</td>
<td>0.224</td>
</tr>
<tr>
<td>25</td>
<td>0.153</td>
<td>0.109</td>
</tr>
</tbody>
</table>

Arrhenius plots were then constructed and are shown in figures (6 and 7) for formula I and A, respectively. The linearity of the curves indicates their utility in predicting the rate of degradation at lower temperatures.

**Figure (5): Degradation curve of granular rifampicin suspension (formula I) at different temperatures**

**Figure (6): Arrhenius plot for expiration date estimation of rifampicin suspension formula I at 25°C.**

**Figure (7): Arrhenius plot for expiration date estimation of rifampicin suspension formula A at 25°C.**

The rate constants at 25°C, obtained from those plots for ready-to-use (formula A) and granular suspension (formula I) were equal to 0.153 x 10⁻³ and 0.109 x 10⁻³ (days⁻¹) respectively. Since the degradation of the drug followed first order kinetics, the expiration date t10% at 25°C could be calculated using the following equation:

\[
t_{10\%} = \frac{0.105}{K_{25^\circ C}}
\]
The expiration dates for formula A and formula I were 1.8 and 2.6 years respectively, indicating that rifampicin is more stable when it is prepared as granulated powder for reconstitution, as also indicated by Patankar and Bajaj [11].

References
Synergistic Effect of Potassium Clavulanate in Combination with Cefamandol and Ceftazidime on β-Lactamase, Extracted From Resistant E.coli

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*Ministry of Industry and Mineral
**Ministry of Industry and Mineral

Abstract
The aim of this study was to evaluate in-vitro activity of Cefamandol and Ceftazidime, in combination with potassium clavulanate against 10 uropathogenic E.coli isolated from patients with chronic complicated urinary tract infections (UTIs), these isolates were identified by the Api identification systems. The antimicrobial susceptibility tests were determined by Kirby-Bauer method and the minimum inhibitory concentrations of Cefamandol and Ceftazidime, were determined, by tube method. These isolates were resistant to Ampicillin (Amp), Amoxicillin (Amo), Carbenicillin (Cb), Ticarcillin (Tic), Amoxicillin Potassium Clavulanate {Augmentin}, (AmoCA), Ticarcillin Potassium Clavulanate {Timentin} (TicCA), Cefamandol (Cfm) and Ceftazidime (Cfz), also resistant to other antibiotics, such as Tetracycline, Chloramphenicol, Trimethoprime and (50% of the isolates were resistant to Nalidixic acid and Rifampicin). Transfer of plasmids by direct conjugation experiments were performed by mating 10 strains with recipient strain E.coli K12C 600 Rif or Nal resistant, and cell free β-lactamases were prepared and detected by macro-iodometric method. The activities of each cell free β-lactamases extract against Cfm and Cfz were determined by disks diffusion method (microbiological Masuda method) and by macro-iodometric method. The activity of β-lactamases was inhibited by the addition of Potassium Clavulanate.

Conclusion:
Good effectiveness of Cfm CA and Cfz CA was obtained against resistant strains of E.coli due to complicated urinary tract infection (UTIs).

Key words: β-lactamases, Cefamandol, Ceftazidime, Timentin and Augmentin.
Introduction
Clavulanic acid is a β- lactam; structurally it differs from penicillins in two respects, the replacement of Sulfur in the penicillin thiazolidine ring with oxygen in the clavam oxazolidine ring and the absence of the side chain at position 6. Clavulanic acid a naturally occurring clavam isolated from Streptomyces clavuligerus has poor antibacterial activity but exerts a potent and irreversible inhibitory effect on β-lactamases especially penicillinas by blocking the active sites of these enzymes and is strongly synergistic with most of the β-lactamines in vitro\(^\text{(1,2)}\). Due to this combination, Amoxicillin is protected from degradation and its spectrum is therefore extended to include bacteria normally resistant to amoxicillin and other β - lactam antibiotics \(^\text{(3)}\). In the case of β-lactam resistant bacteria a bacterial enzyme, β-lactamase, cleaves the β-lactam ring and renders the antibiotic inactive-lactamases are a large and diverse group of enzymes in which four clinically relevant classes are known \(^\text{(4)}\). β-lactamases continue to be the leading cause of resistance to β-lactam antibiotics among Gram-negative bacteria. In recent years there has been an increased incidence and prevalence of extended-spectrum β-lactamases (ESBLs), enzymes that hydrolyse and cause resistance to oximino-cephalosporins and aztreonam. The majority of ESBLs are derived from the widespread broad-spectrum β-lactamases TEM-1 and SHV-1. ESBLs have become widespread throughout the world and are now found in a significant percentage of E.coli and Klebsiella pneumoniae strains in certain countries \(^\text{(5,6,7,8)}\). There are also new families of ESBLs, including the cefotaximase(CTX-M) and OXA-type enzymes, cefazidimase, as well as novel unrelated β-lactamases \(^\text{(9,10)}\). The stability of different cephalosporins to the most important β-lactamases was assessed and many clinical studies have shown that up to 75% of the β-lactamases responsible for β-lactam resistance in G-negative bacteria were R-plasmid mediated\(^\text{(11,12)}\). Recently, new fourth generation cephalosporins, such as Ceftizime, Ceftipram, Cefoselis, Cefditoren, Cefozopran \(^\text{(13,14)}\), were introduced into antibacterial chemotherapy and their activities were compared with other β-lactams such as Cefazidime, Imipenem and Carbenapenem, against P.aeruginosa, Enterobacteriaceae (E.coli, Klebsiella pneumoniae) and G-positive bacteria. In addition several drug combinations have been produced which contain both a β-lactam antibiotic and a β-lactam inhibitor; the inhibitor has high affinity for β-lactamases it irreversibly binds to it, and thereby preserves the activity of the β-lactam. Currently, four penicillin inhibitor combinations are in clinical use: Ampicillin + Sulbactam (Unasyn), Amoxicillin + Clavulanate (Augmentin), Ticarcillin + Clavulanate (Timentin) and Pipracillin-Tazobactam (Zosyn) \(^\text{(15,16)}\). Urinary tract infections (UTIs) cause a significant health problem and E.coli has been reported to be the primary pathogen in approximately 80% of cases. E.coli, express structures called adhesions, fimbrae or pili that help them bind to specific tissue \(^\text{(17)}\).

Aim of the Study
The aim of the study is to evaluate, the following combinations, Cefamandol / Clavulanate and Cefazidime/Clavulanate for their in vitro antimicrobial activity against complicated urinary tract infections caused by β-lactamase producer E.coli.

Materials and Methods

Bacterial strains
Standard strains with plasmid – mediated beta – lactamases were used:
1-E.coli K12 (TEM-1 type β- lactamase with isoelectric point 5.4) confer plasmid(R 111) and E.cloacae P99 (11). 2-E.coli K12 (SHV-1 type β- lactamase Piton (type II) Lp 7.7 \(^\text{(11)}\). 3-E.coli K12 C 600 Rif and E.coli K12 C 600 Sensitive to antibiotics \(^\text{(11)}\). 4-Clinical isolates of E.coli. 5-Pure enzyme of Med Labs. All types of antibiotics powder were obtained and kindly provided by SDI. 6-E.coli ATCC 25922 kindly provided by Medical city.

Identification of E.coli
Strains were isolated on MacConkey agar and identified by Api 20 E System (Biomerieux) \(^\text{(18)}\).

Antibiotic susceptibility test (Disk diffusion method)
The resistance pattern for antibiotics were determined by Bauer - Kirby \(^\text{(19)}\) diffusion assay on Mueller Hinton agar (20ml / plate) the inoculum was 104 – 105 bacteria / ml, of 6 hours cultures incubated at 37C0 for 24 hours. The antibiotics used were as follow: Ampicillin30µg, Amoxicillin30 µg, Augmentin (Amo20µg+CA10 µg), Carbencillin 100 µg, Ticarcillin100 µg, Timentin(Tic75µg+CA 10 µg), Cefamandol 30 µg and Cefazidime30 µg, Rifampicin 30 µg, Nalidixic acid 30 µg, Tetracycline 30 µg, Chloramphenicol 30 µg and Cotrimoxazole (Trimethoprine 2.5 µg + Sulfamethazole 22.5 µg). Powders of Cefamandol and Cefazidime were also obtained from (Roussel, Beecham and Sepacia).
Minimum inhibitor concentration (MICs)

This test measures the concentration of an antibiotic necessary to inhibit growth of a standardized inoculum under defined condition. Minimum inhibitory concentrations (MICs) were determined by dilution of different concentration of antibiotics in Mueller – Hinton broth .The tubes were inoculated with a 6 hour incubation cultures , diluted , given a final concentration of inoculum (10^7 – 10^8 CFU/ml ) and incubated at 37°C .The lowest concentration of antibiotic preventing growth and remaining clear (free from microbial growth ) (MIC) was estimated after 18 hours of incubation.

Remaining clear (free from microbial growth) (MIC) was estimated after 18 hours of incubation . As control, fully sensitive E.coli K_12 strain was tested under the same conditions. Table 1 and Table 2 shows normal MICs values and diameters of zone of inhibition according to the method recommended by the National Committee for Microbiology Laboratory Standards (FRANCE) (20).

Transfer of genetic information by direct conjugation method.

Conjugal transfer of 3GC resistant ESBL producing strains was done at 35°C- 37°C in liquid medium (Brain heart infusion (B.H)) or in solid media (Trypticase Soya agar (T.S.A) or Mueller – Hinton (M.H)) using E. coli K_12 C 600 Rif and E.coli K_12 C 600 Nal as recipient. Equal volumes (1 mL) of culture of the donor and the recipient strain (10^8-10^9 CFU/ml) grown with agitation in tryptic soya broth were mixed and incubated statically for 18 hours at 35°C. Transconjugants were selected on M.H agar containing Nalidixic acid (150 μg /ml) or Rifampicin (300 μg /ml) to inhibit the growth of donor and Amoxicillin, Ticarcillin, and Cefazidime to inhibit the growth of recipient strain (11).

<table>
<thead>
<tr>
<th>Table (1) Standard of MICs and diameters (Ø) of zone of inhibition of cephalosporins</th>
<th>Abbreviations</th>
<th>Critical concentrations in μg/ml</th>
<th>Ø of Zone of Inhibition</th>
<th>Potency of disk/μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>First generation</td>
<td></td>
<td>c</td>
<td>C</td>
<td>d</td>
</tr>
<tr>
<td>Cefalothin</td>
<td>Ctn</td>
<td>8</td>
<td>32</td>
<td>18</td>
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<td>Cefaloridin</td>
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<td>Cefalexin</td>
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<td>Second generation</td>
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<td>Cefamandol</td>
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<tr>
<td>Third generation</td>
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<td>Cefotaxime</td>
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<td>Ceftriaxone</td>
<td>Cro</td>
<td>4</td>
<td>32</td>
<td>21</td>
</tr>
<tr>
<td>Cefotiam</td>
<td>Ctm</td>
<td>4</td>
<td>32</td>
<td>22</td>
</tr>
<tr>
<td>Cefmenoxime</td>
<td>Cmx</td>
<td>4</td>
<td>32</td>
<td>21</td>
</tr>
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<td>Cefazidime</td>
<td>Cfl</td>
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<td>32</td>
<td>21</td>
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<td>Cefitoxime</td>
<td>Zox</td>
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<td>Clp</td>
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<td>32</td>
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<td>Hr221</td>
<td>4</td>
<td>32</td>
<td>22</td>
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<tr>
<td>Moxalactam</td>
<td>Mox</td>
<td>4</td>
<td>32</td>
<td>23</td>
</tr>
</tbody>
</table>

MICs: c: Sensitive strains, MIC>C: Resistant strains, C< MIC≤ C Intermediate, Ø≥ D: Sensitive strains, Ø< d Resistant strains d ≤Ø< D Ø=diameter (in mm)
Table (2) Standard values of MICs and diameters (⌀) of zone of inhibition of Penicillins.

<table>
<thead>
<tr>
<th>Penicillins</th>
<th>Abbreviations</th>
<th>Critical concentrations In µg/ml</th>
<th>⌀ of Zone of Inhibition</th>
<th>Potency of disk/µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>c</td>
<td>C</td>
<td>d</td>
</tr>
<tr>
<td><strong>Group A</strong></td>
<td></td>
<td></td>
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<tr>
<td>AMPICILLIN</td>
<td>Amp</td>
<td>4</td>
<td>16</td>
<td>17</td>
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<tr>
<td>AMOXYCILLIN</td>
<td>Amo</td>
<td>4</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>AUGMENTIN</td>
<td>Amc</td>
<td>4</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>TIMENTIN</td>
<td>Tim</td>
<td>128</td>
<td>128</td>
<td>13</td>
</tr>
<tr>
<td><strong>CARBOXYPENICILL</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>CARBENICILLIN</td>
<td>Cb</td>
<td>128</td>
<td>128</td>
<td>15</td>
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<td>TICARCILLIN</td>
<td>Tic</td>
<td>128</td>
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<td>13</td>
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<td><strong>AMIDINOPENICILLIN</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>MECILLINAM</td>
<td>Mec</td>
<td>1</td>
<td>8</td>
<td>23</td>
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<tr>
<td><strong>UREIDOPENICILLIN</strong></td>
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<td>AZLOCILLIN</td>
<td>Azl</td>
<td>16</td>
<td>128</td>
<td>19</td>
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<tr>
<td>PIPRACILLIN</td>
<td>Pip</td>
<td>16</td>
<td>128</td>
<td>20</td>
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<tr>
<td><strong>MONOBACTAM</strong></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>AZHEREONAM</td>
<td>Atm</td>
<td>4</td>
<td>32</td>
<td>23</td>
</tr>
</tbody>
</table>

MIC ≤ c: Sensitive strains, MIC > C: Resistant strains, C ≤ MIC ≤ C Intermediate, ⌀ ≥ D: Sensitive strains, ⌀ < D: Resistant strains D ≤ ⌀ ≤ D ⌀ = diameter (in mm).

**Extraction of β- lactamase.**

Cell free beta–lactamases were prepared from strains known to be good producers of the desired enzymes, (β–lactamases, type TEM-1 E.coli R111 and SHV-1 E.coli453, R-plasmid mediated enzymes) and β–lactamase from E.cloacae P99( cephalosporinase) as reference. Crude enzymes were also prepared from test isolates of E.coli. Bacterial cultures were grown aerobically at 37°C in Brain Heart Infusion broth (Difco) over night. A 200 ml flask of the same broth was then inoculated with 2ml of the culture, incubated at 37°Cfor 4 hours, the cells were harvested by centrifugation, washed twice with buffer phosphate pH 7 and disrupted with ultrasound (soniprep 150HSE) at 20KHZ. To remove cell debris, the crude extracts were centrifuged; the supernatants were collected in small sterile vials under aseptic conditions (11).

**Detection of β–lactamase by Macro - iodometric method.**

1% of agarose and0.5% of starch were dissolved in120ml of buffer phosphate and boiled. 18 mg of penicillin G powder and0.8ml of iodine solution were added at 40°C, the medium was shaken and distributed in aliquots of 20ml in Petri dishes, 5 well were made in each plate, the enzymes were applied in each well and the zones of decolorization were observed 1-18hours at 4°C (21).

**Assessment of stability of β–lactams to cell-free β–lactamases (22,23,24)**

The activity of each cell free β–lactam extract against each β–lactam antibiotic was determined by the microbiological method (Masuda G., et al. 1976,modified by Labia R. Barthelemy M. 1979). The surface of a Muller Hinton agar was seeded with a suspension of β–lactam sensitive indicator E.coli ATCC 25922. Four discs containing β–lactams under test were placed near filter paper discs; each impregnated with 30µl of the enzymatic extract. The plates were incubated at 37°C for 18hours, the β–lactamase activity was observed like half moon zone of inhibition. Unchangeable inhibition zones demonstrate stability of the antibiotic to the enzyme.

**Inhibition by Cefamandol or Cefazidime /Clavulanate Modified iodometric method (21).**

Modified iodometric method (Labia R., Barthelemy M., 1979),was used without incorporation of penicillin G in the medium, five wells were made in the plate in which 10µl of enzyme extract , 30 µl of potassium clavulanate and 30µl of Cefamandol or Cefazidime were added. The results were noted after 4-18 hours at 4°C, absence of decolorization zone indicated positive reaction.
Masuda microbiological method
Ten clinical isolates were screened for \( \beta \)-lactamase inhibitor using 10µl potassium clavulanate in combination with 30 µl of Ceftazidime or Cefamandol. Sensitivity discs containing Ceftazidime or Cefamandol and a filter disc incorporated with 30µl enzyme and 10µl potassium clavulanate were placed on agar plate on which a bacterial suspension of sensitive E.coli (standard) was spread the inoculum was 104 – 105 CFU / ml, of 6 hours cultures at 37°C for 24 hours according to the method recommended by the National committee for microbiological Laboratory standards (25).

Results and Discussion

Disk agar diffusion test (Susceptibility test)
According to the results of Susceptibility test. The resistance patterns of E.coli RIII (TEM-1 beta-lactamase) and E.coli K12 (SHV-1) type \( \beta \) - lactamase Pitton (type II ) I.p 7.7 were compared with ten strains they were resistant to Ampicillin , Amoxicillin , Carbenicillin Pipracillin . Augmentin , Timentin , Cefamandol and Ceftazidime . They were also resistant to other antibiotics such as Tetracycline ,Chloramphenicol and Trimethoprim and (50%) were resistant to Rifampicin and Nalidixic acid . The results indicated dissemination of resistance among clinical isolates of E.coli in Iraq table 3.

Detection of \( \beta \)-lactamases
This test is based on the reaction of the (oic) acid of penicillin with iodine . \( \beta \) - lactamase hydrolyze penicillin to penicilloic acid , which in turn react with iodine , the presence of \( \beta \) - lactamase in a test system was shown by decolorization of starch – iodine complex . The results of detection of \( \beta \) - lactamases by iodometric method were positive for 10 strains compared with standard negative E.coli K12 C 600 Rif and positive \( \beta \) – lactamases R111 (TEM-1), presented in Fig 1.
Minimum inhibitory concentrations

The inhibition of beta-lactamase production by potassium clavulanate has been demonstrated with many strains of bacteria, this effect potentiates the action of many beta-lactams, such as Ampicillin, Amoxicillin, Carbenicillin and Ticarcillin. Many clinical reports of combination of Amoxicillin with Clavulanic acid (Augmentin) have been encouraging, in urinary tract infections due to beta-lactamase producing organisms type TEM and SHV, whilst Amoxicillin alone had no effect, the addition of Clavulanic acid (as salt) dramatically change the half moon inhibition zone to complete inhibition zone (3,4,11).

Our investigations indicated resistant phenotype of Augmentin and Timentin; the diameters of zone of inhibition ranged from (3.5mm-7.5mm) for Amc and (5.5mm-9.5mm) for Tim, while The standard diameters zones of inhibition were for Amc(14-21mm) and for Tim is(13mm). The critical normal MICs of Augmentin and Timentin were (4-16 µg /ml), (128 µg /ml) respectively.

The minimum inhibitory concentrations were studied for ten clinical isolates of E.coli in comparison with standard resistant strains, TEM-1 beta-lactamase coded for plasmid R111, E.coli K12 SHV-1 beta-lactamase Pitton (type II) coded for plasmid 453 Lp 7.7, E.cloacae P99 cephalosporinase Lp 8.3 (France) and E.coli ATCC 25922 (Medical city hospital) sensitive strains as references, the MICs of Cfm, Cfz, were very high, the range of MICs for Cfm was 512 - 2048 µg/ml for Cfz 64 - 32 µg/ml. These results are indicated in Table 4.

Table (4) Minimum Inhibitory Concentrations OF Four Antibiotics Towards Ten Uropathogenic E.coli Comparing with Standard Strains

<table>
<thead>
<tr>
<th>No. of Isolate</th>
<th>E.coli</th>
<th>Cefamandol</th>
<th>Ceftazidime</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2,3</td>
<td></td>
<td>512</td>
<td>'64</td>
</tr>
<tr>
<td>4,5,6</td>
<td></td>
<td>1024</td>
<td>32</td>
</tr>
<tr>
<td>7,8,9,10</td>
<td></td>
<td>2048</td>
<td>32</td>
</tr>
<tr>
<td>E.coli(453)**(\text{SHV-1(7.7)}^*)</td>
<td>16</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>E.coli(R111)**(\text{TEM-1(5.4)}^*)</td>
<td>32</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>E.cloacae(P99)**(\text{SHV-1(8.3)}^*)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Isoelectric point , ** ephalosporinase

Inhibition of beta-lactamases

Figure 2 shows comparisons between antibiotic-enzyme interactions, by the highly sensitive double disk technique which demonstrated hydrolysis of Ceftazidime and Cefamandol by beta-lactamase-producing E.coli. The enzymes obtained from 10 strains hydrolyzed, Cefamandol and Ceftazidime, but were highly stable to all beta-lactamases tested when combined with potassium clavulanate. Enzymes extracted from E.coli standards harboring plasmid R111 TEM-1 or SHV-1 harboring plasmid R 453 beta-lactamases were inhibited with potassium clavulanate when combined with Amoxicillin or Ticarcillin (Fig 3 A, B) however beta-lactamase of E.cloacae was not affected by Augmentin and inhibited by Ticarcillin and hydrolyzed all cephalosporins represented in Fig 4. In contrast beta-lactamase under test were highly resistant to AmoxCA, TicCA, Fig 5: show inhibition of enzymes by iodometric method.
It was found that copies of the genes for ampicillin, ticarcillin, tetracycline, and chloramphenicol resistance could be transferred by direct conjugation method from donor cell to recipient cell. The results were presented as follow:

**Figure (2):** Antibiotic – enzyme interactions, by the highly sensitive double disks technique (25) demonstrated the inhibition of β-lactamases by clavulanic acid. A and B: 1 and 2 -disks of Cefazidime (Cfz) 30µg, and Cefamandol (Cfm) 30µg, 3 and 4 disks at a distance of 2 cm from Cfz and Cfm impregnated with (10µl) of enzyme(β-lactamase)5 and 6 disks impregnated with 10µl enzyme + 10µl Clavulanic acid (CA).

**Figure (3) A:** Susceptibility disk diffusion method shows the activity of Augmentin (Am) and Timentin (Tm) against plasmid – mediated β-Lactamase produced by E.coli SHV – 1 Pitton type II(P453)(11)

**Figure (3) B:** Action of inhibitor clavulanic acid (CA) on the activity of standard β-lactamase extracted from E.coli SHV-1 Pitton type 1: Ampicillin , 2: β-Lactamase 3:β-Lactamase+clavulanic acid.

**Figure (4):** Hydrolysis of Cephalosprins by Cephalosporinase (β-Lactamase), extracted from E.cloacae P99 (According to Sirot, D,1983)

**Figure (5):** Inhibition of β-Lactamase of Clinical Isolates (E.coli) By Modified Iodometric Method Comparing With Standard Strain of E.coli ATCC 25922 β-Lactamase Non Producer

**Direct conjugation method**

It was found that copies of the genes for ampicillin, ticarcillin, tetracycline, and chloramphenicol resistance could be transferred by direct conjugation method from donor cell to recipient cell. The results were presented as follow:
Ten strains transferred resistance to Ticarcillin, Cefazidime, and other antibiotics after mating for 18 hours, transconjugants derived from these strains produced β-lactamases, these results suggested that all strains bear plasmids and produce extended spectrum β-lactamases capable of hydrolyzing and inactivating a wide variety of β-lactams including third generation cephalosporins and Aztreonam, sensitive to imipenem, these results were similar to the studies of Rodrigues C, et al., Chaudhary U, and Kurokawa, H et al. 56,27,28.

### Conclusions

The clinical isolates in this study were very resistant to Augmentin, Cefamandol and Cefazidime comparing with standard TEM-1 and SHV-1 (pasmidic pencillinases). E. cloacae P99 is very resistant to Cefalothin, Cefamandol, Cefotaxime, Cefazidime standard strain which produce β-lactamase (ESBLs) enzymes that hydrolyze and cause resistance to oxyimino-cephalosporins and aztreonam. The majority of ESBLs are derived from the widespread broad-spectrum β-lactamases TEM-1 and SHV-1. ESBLs have become widespread throughout the world and are now found in a significant percentage of E. coli and Klebsiella pneumonia strains in certain countries. 8,7,8,10.

The increasing emergence cephalosporin-resistant E. coli has led to concern about the use of various combination therapy. A good in vitro response was observed in our clinical uropathogenic E.coli when Cfm and C fz were mixed with different concentration of potassium Clavulanate as inhibitor they were effective and safe for the treatment of UTIs caused by β-lactamases (Cefazidimase) producing complicated strains.

### REFERENCES


22. 22. – Ma, Y; Li, J-Y; Yao, L; Zhang, L; Hu, C-Q; Jin, S-H; Zhonghua-Yi-Xue-Za-Zhi. Antimicrobial resistance of Escherichia coli isolates collected from inpatients and outpatients] 2003 Jun 25; 83(12): 1046-8


Micro encapsulation of Naproxen By Complex Coacervation and Aqueous Colloid Dispersion  Part (1)

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**Department of Department Pharmaceutics, College of Pharmacy, University of Baghdad, Baghdad-Iraq

Abstract

Naproxen is a non steroidal anti inflammatory and antipyretic drug which has local irritation effect on the stomach, and unpleasant taste, besides bad flowability and light sensitivity. The drug was prepared as microcapsules by complex coacervation method using acacia-gelatin coating materials, and aqueous colloid polymer dispersion method (ACPD), using ethyl cellulose and sodium alginate coating materials. The results indicated that microcapsules prepared by 2:1 core:wall ratio is the best for both methods, with an average encapsulation efficiency (75%) and average yield (90%). Moreover the drug release was affected mainly by core:ratio, pH environment and method of microencapsulation.

Key words: Naproxen, Microcapsule, Aqueous colloid polymer dispersion

Introduction

The principle of microencapsulation is formation of a thin coating of wall material around the substance, therefore making the particles more desirabl in terms of physical and chemical properties 1,2, the quantity of drug incorporation as core material vary from 20-95% 3. Naproxen is aryl acetic acid group derivative of non-steroidal anti inflammatory drugs widely used in rheumatoid arthritis and ankylosing spondylitis 4. The main side effects of this drug are GIT disturbances and bitter taste. The goals of this study are to mask the irradiation effect of the active drug to the GIT, and improve physical properties of the drug like flowability and light sensitivity

Experimental

Materials:

Naproxen powder supplied by Samarra” Drug Industry (SDI), acacia, gelatin, ethyl cellulose, sodium alginate, calcium chloride, formaldehyde, from Riedel De-Haen AG, Seelze, Hannover, Germany

All other reagents are of an analytical grade.

Instruments:

sartorius balance, type 1219 MP3, electrical stirrer Ike - Werk type Re- 16, (Germany), oven, mammert 854 Schwa Bach, (Germany), microscope, Olympus CX21 Tokyo Japan, Cintra 5 GBC, UV-Visible spectrometer, dissolution apparatus Erweka USP DT6 Hansen (Germany), water bath pH-meter (W-Germany).

Preparation of naproxen microcapsules (complex coacervation)

The microcapsules were prepared by incorporating naproxen powder in 50 ml of 2% w/w acacia solution previously heated to 40°C, then 50 ml of gelatin solution 2% w/w at 40°C was added and maintained at 250 rpm stirring speed for 50 minutes adjusting the pH of mixture to 4 with few drops of diluted HCL (0.1M), the formation of microcapsules can be watched microscopically , then 10 ml of formaldehyde solution was added with continuous stirring for 10 minutes, cooling in ice bath, filtered the microcapsules formed using three portions of 100 ml isopropyl alcohol and then the wetted microcapsules were dried before the free flowing powdered microcapsules obtained 5.

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Received 25-12-2005
Accepted 25-7-2006
Preparation of naproxen microcapsules (ACPD method)
The drug was dispersed in 50ml. of 2% w/w aqueous sodium alginate solution heated to 40°C, then 50ml. of 30% w/w ethyl cellulose prepared at room temperature was added with continuous stirring. (the weight of naproxen added depend on core:wall ratio of microcapsules prepared). The mixture was allowed to drop through modified separatory funnel into gently agitated calcium chloride (1% w/w), the gelled beads were separated after 2 minutes by vacuum filtration, rinsed with distilled water and allow to dry at 40°C over night. All the microcapsules prepared by both methods were of 1:2 ,1:1 and 2:1 core : wall ratio which corresponds to 33.3% ,50% and 66.6% drug content respectively.

Dissolution behaviour
For all types of microcapsules prepared under sink conditions, the dissolution behaviour of naproxen was carried out using 100mg, equivalent dose in 900ml of different dissolution medium pH (1.2,4.2 and 6.8 ) at 37 ± 0.5C and constant stirring speed of 50 rpm. Then filtered samples were taken for analysis at different specified time intervals, The absorbance of each sample was determined spectrophotometrically at 272,330 and 271 nm, respectively.

Results and Discussions
Preparation of microcapsules:
Table 1. (A and B) illustrates the yield percent and the encapsulation efficiency of naproxen by different preparation methods and core: wall ratios, it was found that coacervation method gave 75-85% yield compared with 86-93% given by ACPD method, while encapsulation efficiency was 48-82% and 27-70% for complex coacervation and ACPD method, respectively.

<table>
<thead>
<tr>
<th>Core To Wall ratio</th>
<th>Microencapsulation yield</th>
<th>% Yield</th>
<th>Drug loading %</th>
<th>Microencapsulation efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:1</td>
<td>12</td>
<td>85.4</td>
<td>66.6</td>
<td>81.9</td>
</tr>
<tr>
<td>1:1</td>
<td>8</td>
<td>77.5</td>
<td>50</td>
<td>75</td>
</tr>
<tr>
<td>1:2</td>
<td>6</td>
<td>75</td>
<td>33.3</td>
<td>48.5</td>
</tr>
</tbody>
</table>

Table (1) Effect of variation of core to wall ratio of naproxen microcapsules on the microencapsulation by:-A- Complex coacervation method

<table>
<thead>
<tr>
<th>Core to wall ratio</th>
<th>Microencapsulation yield</th>
<th>% Yield</th>
<th>Drug loading %</th>
<th>Microencapsulation efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:1</td>
<td>70</td>
<td>66.6</td>
<td>93</td>
<td>14</td>
</tr>
<tr>
<td>1:1</td>
<td>60</td>
<td>50</td>
<td>85</td>
<td>8.5</td>
</tr>
<tr>
<td>1:2</td>
<td>27</td>
<td>33.3</td>
<td>86</td>
<td>6.5</td>
</tr>
</tbody>
</table>

The results indicated that best encapsulation efficiency was in complex coacervation compared with ACPD method, which may be attributed to the ability of polymer used to encapsulate the drug. On the other hand, the results revealed that encapsulation efficiency appeared to be core weight dependent, Higher percent of drug loading was shown with 2:1 core wall ratio. These results are consistent with the results obtained in case of metronidazol and chloramphenicol microcapsules, moreover the appreciable amounts of microcapsule gained (86 - 93%) by ACPD method may be referred to the compatibility between naproxen and polymer used, since both of them are acidic in nature.

Dissolution behaviour of microcapsules:
Figure 1 and 2 shows the release profiles of naproxen from 2:1 core wall ratio microcapsules for both methods of preparation at pH 6.8 and 1.2 respectively.
It was found that in complex coacervation method, the release was faster than in ACPD method at pH 6.8, since 50% of drug release took over 8 minutes for the first method compared with 75 minutes for the later; these results are consistent with the results obtained by microencapsulation of diclofenac sodium (10) and protein (1).

The drug release at pH 1.2 decreased significantly for both methods (not more than 30% at first two hours); this behavior may be attributed to the nature of naproxen dissolution at low pH (12).

On the other hand, the effect of core wall ratio on the release of naproxen from microcapsules was shown in figures 3 and 4. It was seen that high significant difference (p < 0.001) in the percent drug release after 120 minutes between 2:1 and 1:2 core wall ratios (coacervation method), the cause is referred to both drug content and solvent penetration, this in consistent with the results obtained by the study of dissolution of diazepam microcapsules (13).
methods constructed for both coacervation and ACPD
these microcapsules, figures 5 to 10 were
to study the effect of different
ratios on the release of naproxen from
tating core-wall ratio respectively.
In an attempt to study the effect of different
ph-medium on the release of naproxen from
these microcapsules, figures 5 to 10 were
constructed for both coacervation and ACPD
methods.

Figure (4): Effect of varying core-wall
tions on the release of naproxen from
microcapsules prepared by ACPD method
in 0.1N HCl pH 1.2.

The same results were obtained from
microcapsules prepared by ACPD method.
since 40,24 and 20% of drug released from
microcapsules from 1:2, 1:1 and 2:1 core wall
ratio respectively.

Figure (5): Effect of pH on the release of
naproxen from 1:2 core-wall ratio
microcapsules prepared by complex
coaervation method.

Figure (6): Effect of pH on the release of
naproxen from 1:1 core-wall ratio
microcapsules prepared by complex
coaervation method.

Figure (7): Effect of pH on the release of
naproxen from 2:1 core-wall ratio
microcapsules prepared by complex
coaervation method.
The results showed that there are high significant differences (p < 0.001) in t50% drug release from different core wall ratios prepared by coacervation method at pH 1.2 and 4.2 in comparison with pH 6.8 (microcapsules prepared by coacervation method at pH 1.2 and 4.2).

The same results were recognized for the same core: wall ratios microcapsules prepared by ACPD method, these results are the same results obtained in the evaluation of sulfonamide ibuprofen and mefenamic acid microcapsules.

**Conclusion**

Based on the results obtained, one may concludes the followings:

a. Both complex coacervation and ACPD methods are valid for microencapsulation of naproxen.

b. The dissolution behaviour of naproxen from microcapsules is affected by pH medium, core:wall ratio and preparation methods.

c. The results obtained in this study could be used to formulate naproxen in many microencapsulated dosage forms.

**References**


9. El- Gibaly I., Safwat S.M. and Ahmed M.O., microencapsulation of ketoprofen using


The Changes in Sex Hormones in Female Working in Batteries Manufacturing Plant

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** Department Clinical Pharmacy, College Of pharmacy, University of Baghdad, Baghdad-Iraq

Abstract

Lead has toxic effects on reproduction of both male and female. It can cause decreased sex drive, infertility and abnormal menstrual cycle in women. This study was designed to evaluate the effect of exposure to lead in batteries female workers on sex hormones level in the serum. Thirty nine (39) female workers (volunteers) in Iraqi Batteries Manufacturing Plants, Al-Waziriya / Baghdad were participated in this study. They are classified into 3 groups, first group included fourteen (14) female that have been employed for 1-7 years, second group included thirteen (13) female that have been employed for 8-14 years, third group included twelve (12) female have been employed for 15-22 years and fourteen females were included as the control. Blood lead level, serum FSH, LH, prolactin and total testosterone were measured and compared for all subjects. The results indicated that mean of blood lead levels (BLL), testosterone levels were highly significant in all worker groups compared to the control (p<0.005). Prolactin levels in group I and FSH in group III were significantly higher than that in control (P<0.005) and (P<0.05) respectively. LH levels in groups II and III were significantly higher than that in control (P<0.05, P<0.005 respectively). High incidence of hirsutism (48%) and miscarriages (50%) were observed in worker groups compared to control (11%). The results indicated that there are hormonal changes in female workers exposed to lead associated with increased incidence of hirsutism and miscarriages compared to non exposed females.

Key words: Lead, Sex hormones, hyperandrogenemia

الخلاصة

تسعون وثلاثون امرأة عاملة في معمل صناعة البطاريات في بغداد شاركن في هذه الدراسة كمتطوعات قسمت العاملات إلى ثلاث مجموعات اعتماداً على فترة التعرض للرصاص.

1. المجموعة الأولى: تشمل (14) عاملة وفترة الخدمة تتراوح بين (1-7) سنوات وبعمر (43-54) سنة.
2. المجموعة الثانية: تشمل (13) عاملة وفترة الخدمة تتراوح بين (8-14) سنة وبعمر (40-53) سنة.
4. المجموعة الرابعة: مجموعة السيطرة وتشمل (14) امرأة متوسط عمر (24-39) سنة.

وجد في هذه الدراسة أن هناك تغير في مستويات الهرمونات الجنسية عند النساء العاملات عند مقارنتها بمجموعة السيطرة. وكالنُكُل أن مستوي الهرمونات الجنسية في المصل يزداد عند العاملات اللواتي تعرضن للرصاص لفترة طويلة ما هورمون خص办公楼 لفترة طويلة (البرولاكتين) فإنه يزداد فقط عند التعرض لفترة قصيرة للرصاص. كذلك هورمون التستوستيرون الكلي يزداد عند النساء العاملات متصاحباً مع زيادة في نسبة الشعرية والاضطاعات عند العاملات مقارنة بجماعة السيطرة.

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Received 21-11-2005
Accepted 7-10-2006
Introduction

Some scientists cautioned the Romans of the danger of inhaled fumes from lead smelting (4). With the industrial revolution, lead poisoning became a common occupational problem. The reproductive effects of lead poisoning were also noted by the turn of the century and many articles describe the high rate of stillbirths, infertility and abortions among women in the pottery industry, or who were married to pottery workers (2).

The most important route of absorption in occupational setting is through inhalation of lead dust and fumes. In addition, workers may eat, drink or smoke in lead-dust-contaminated areas resulting in some ingestion as well. Storage battery manufacture involves considerable exposure to lead oxide dust, in addition to fumes from welding of battery connector (o).

Lead can cause decreased sex drive and infertility in women. In addition, it can cause abnormal menstrual cycles (dysmenorrhea, menorrhagia and amenorrhea), premature birth, spontaneous miscarriages, and stillbirths. The incidence of polycystic ovary syndrome (PCOS), prolonged and abnormal menstruations, hypermenorrhea was significantly higher in the lead exposed group (female workers of lead battery plants) than in controls (4). This study was designed to evaluate the hormonal changes (LH, FSH, prolactin and testosterone) in women whom exposed to lead in batteries manufacturing plants through inhalation and direct contact with active constituent of batteries.

Subjects and Methods

This study was carried out on female workers employed in Iraqi batteries manufacturing plants, Babylon 1 and 2 in Al-Waziriya / Baghdad for the period of three months from January to April of 2005.

Thirty nine (39) female workers were participated in this study, they work 6 hours per day every other day and had been employed for at least 1 year in the plant.

The subjects were classified into 3 groups according to the duration of exposure to lead (employment in the plant) as follow:

Group I includes fourteen (14) female that have been employed for 1-7 years with range age (24-50) years (29.2±7.2) years.

Group II, includes thirteen (13) female that have been employed for 8-14 years with range age (30-55) years (37.9±7.9) years.

Group III includes twelve (12) female that have been employed for 15-22 years with range (32-52) years (41.7±6) years.

Fourteen healthy women, not exposed previously to lead with age range (24-50) years (29±7.1) were utilized as control.

Individual questioner protocol was followed for all women concerned with gynecological and obstetrical history including married or not, age of marriage, number of children, type of delivery (normal or caesarean section), number of miscarriages, growth of their infants, if they work during pregnancy, regular or irregular menstrual cycles, if they have amenorrhea, dysmenorrhea or menorrhagia. Appropriate day for each female (between 2nd and 5th day of menstrual cycle) was selected to consider follicle phase for FSH and LH assay, and female were advised to fast 12 hr. before sampling, for appropriate analysis of prolactin. Blood samples (14 ml) were drawn from each patient and control by vein puncture left to clot and serum was separated by centrifugation.

Blood lead levels were measured using the slotted quartz tube method. LH, FSH, prolactin levels in serum were analyzed using radioimmunoassay methods(6,7,8) while testosterone was assessed according to the method of Abraham et al (9). All these kits supplied by Immunotech, A Beckman coulter company (France).

Independent t-test was used to examine the difference in the mean of control and workers, also the differences among worker groups themselves. P-values < 0.05 were considered as significantly different. Pearson correlation (r) was performed to find relationship between exposure time and testosterone levels.

Results

Table (1) shows the ages and occupational (exposure) periods of female worker. The number of married were 3, 6, 7 in I, II, III groups respectively. The mean age of control group was 29.1±7.1 years and the number of married was 5.

Table (2) shows mean blood lead levels in all groups of workers are significantly (p<0.005) higher than that of the control group. Groups II and III workers have significantly higher blood lead levels than that of group I workers (p<0.05). Serum total testosterone levels in all three worker groups were significantly higher in comparison with that of the control group (P<0.005). Total testosterone level for group I workers was the highest one. However group I workers have a serum total testosterone level that was significantly higher than that of groups II and III workers (P<0.05). Mean prolactin level in group I workers shows highly significant increase compared to that of the control group (P<0.005). While mean prolactin levels in groups II and III were non-significantly elevated compared with the control group (P>0.05). Group I workers have prolactin levels

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significantly higher than that of groups II and III of workers (P<0.05). Mean FSH level of group III workers was significantly elevated (p<0.05) in comparison with that of the control group. While mean FSH levels of workers group I and II were non-significantly elevated (P>0.05) compared to control group. FSH levels show non significant difference among worker groups themselves. Mean LH level in group II workers was elevated significantly in comparison with that of the control group. LH level in group III workers shows a highly significant increase compared with control group (P<0.005). Furthermore mean LH level in group I workers was non-significantly elevated (P>0.05) compared to that of the control group. LH levels show non significant (P>0.05) difference among worker groups themselves. There are non significant differences among LH/FSH ratio in all three groups of workers and control group (P>0.05). Also LH/FSH ratio show non significant (P>0.05) difference among worker groups.

Table (1) Demographic data of workers and control women.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Age (mean ±SD) (years)</th>
<th>Occupation period (mean ±SD) (years)</th>
<th>Number of married</th>
<th>Number of unmarried</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>29.2±7.2</td>
<td>3.6±1.9</td>
<td>3</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>II</td>
<td>37.9±7.9</td>
<td>11.1±1.4</td>
<td>6</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>III</td>
<td>41±6</td>
<td>18.2±2.8</td>
<td>7</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Control</td>
<td>29.1±7.1</td>
<td>-</td>
<td>9</td>
<td>5</td>
<td>14</td>
</tr>
</tbody>
</table>

Table (2) Serum levels of lead, testosterone, prolactin, follicle stimulating hormone (FSH) and luteinizing hormone (LH) in females working in battery industries.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead (µg/dl)</td>
<td>13.4±4.5</td>
<td>21.4±6.7** a</td>
<td>31.6±7.2** b</td>
<td>29.7±7.3** b</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>0.156±0.5</td>
<td>0.458±0.15**a</td>
<td>0.35±0.13**b</td>
<td>0.28±0.1**b</td>
</tr>
<tr>
<td>Prolactin (ng/ml)</td>
<td>8.5±4.1</td>
<td>19.7±11.9** a</td>
<td>10.7±7.2** b</td>
<td>11.7±8.1** b</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>6.9±2.7</td>
<td>13.3±14.8**a</td>
<td>10.9±8.4**a</td>
<td>14.4±13.6**a</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>8.8±3.3</td>
<td>11.1±3.7**a</td>
<td>14.7±10.4**a</td>
<td>17.3±9.1**a</td>
</tr>
<tr>
<td>LH/FSH</td>
<td>1.2±1.64</td>
<td>0.83±0.45**a</td>
<td>1.3±1**a</td>
<td>1.2±0.85**a</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD
*P<0.05 significant difference from control group
**P<0.005 highly significant difference from control group
Values with different letters (a, b) are significantly different (P<0.05).

Figure (1) shows the correlation between time of lead exposure and serum testosterone levels in female workers. A negative correlation was found between time of lead exposure and serum testosterone levels (r=-0.43), (P<0.05).

In table (3) all workers groups have percentage of hirsutism (17.9%) and miscarriages (50%) higher than that in control group. In table (3) all workers groups have percentage of hirsutism (17.9%) and miscarriages (50%) higher than that in control group.

Table (3) Distribution of female workers with hirsutism and miscarriages.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group I No. (%)</th>
<th>Group II No. (%)</th>
<th>Group III No. (%)</th>
<th>Total</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hirsutism</td>
<td>2(14)</td>
<td>3(25)</td>
<td>7(17.9)</td>
<td>0(0)</td>
<td></td>
</tr>
<tr>
<td>Miscarriages*</td>
<td>1(33)</td>
<td>3(50)</td>
<td>4(57)</td>
<td>8(50)</td>
<td>1(11)</td>
</tr>
</tbody>
</table>

25
The results indicated that mean of blood lead levels (BLL) were significantly higher in all worker groups compared to the control group (P<0.005). This may be due to long-term exposure to lead oxide which is used in batteries manufacturing plant. The workers practice in improper environment, they were not wearing face mask, cloves with bad ventilation inside plant rooms, in addition to that workers were eating inside the manufacturing rooms, which means they did not follow the conditions of occupational safety. The workers in group I had blood lead levels significantly lower than that in groups II and III (P<0.05); this may be due to longer exposure time in groups II and III than that for group I.

It has been reported that patients with blood lead levels less than 45µg/dl do not require chelation therapy. The mean blood lead levels in most female workers are less than that reported in previous studies (11,12), because females in present study are working every other day in the plant since 1.5 years, because of shortage of raw materials.

The mean serum testosterone levels in all worker groups were significantly higher than that of control group (P<0.005) (table 2). Figure (1) demonstrated a negative correlation between time of exposure to lead and serum testosterone levels in worker groups. The data showed an inverse relationship between time of lead exposure and serum testosterone levels, which may need further investigations to explain the correlation. The same results have been reported by Sokol-Rz (13), where the rats treated with various doses of lead acetate for more than 1 week exhibited a significant increase in Gonadotropin releasing hormone (GnRH) mRNA, but with attenuation of the increase at higher concentrations of lead with increased duration of exposure. They concluded that the signals within and between the hypothalamus and pituitary gland appear to be disrupted by long-term lead exposure.

LH stimulates theca cells of ovary to produce testosterone in females (14). However, in this study, it has been found that as LH level increases, testosterone level decreases (table 2), indicating that elevation of testosterone level in female workers may be extra ovarian. However, most of studies concerning lead were applied on animal models so it is difficult to compare present findings with their results. Group I have a high mean prolactin levels which are significantly higher than that of control group (P<0.005); while mean prolactin levels in group II and group III were not significantly different from control group (P>0.05) (table 2). Prolactin release from the pituitary is under tonic inhibitory control from hypothalamus-derived dopamine or prolactin inhibitory factor (PIF). Thyrotropin-releasing hormone (TRH) in turn is stimulatory to prolactin release. Estrogen can directly sensitize the pituitary to release prolactin (15); The elevation of prolactin levels in worker groups could be attributed to that lead affects dopaminergic control of prolactin secretion from pituitary gland, according to previous study on rats (16).

The results demonstrated that only group III workers have mean serum FSH level significantly higher than that of the control group (P<0.05) (table 2). The elevation in serum FSH also reported by Ng et al. (17) where significantly higher serum FSH and serum LH levels were observed in lead-battery male workers, during less than 10 years exposure period, whereas those exposed for 10 years or more showed normal serum LH and serum FSH concentrations. However, Vivoli G et al. (18) reported negative relationships between blood lead level and LH and FSH in males with lead levels higher than 9µg/dl.

Group I workers are presented with serum LH not significantly different from that in control group (P>0.05) while group II and III have mean serum LH significantly higher than that in control group (P<0.05, P<0.005 respectively) (table 2). This elevation in serum LH levels are in agreement with that reported by Rodamilans et al (19) who reported that in lead-smelter workers, serum LH levels are significantly raised, as compared with controls.
Furthermore, Yen suggested that basal LH years 35 and continue to climb until several years after onset of menopause due to decreased negative feedback controls on production. In female rats exposed to lead prepubertally delay vaginal opening with more severe reproductive disruption, accompanied by suppression of circulating estradiol. Effects on circulating sex steroids were accompanied by variable effects on circulating LH levels, pituitary LH, and pituitary LH beta-mRNA. Increase in hypothalamic levels of GnRH mRNA and an increase in pituitary levels of LH mRNA and pituitary stores of LH in lead exposed animals. This increase due to lead disrupts the reproductive axis by interfering with feedback mechanisms at the hypothalamic and pituitary levels.

In this study the results indicated that lead exposure has no effect on LH/FSH ratio in female workers. Elevated basal LH with an LH/FSH ratio > 2 and some increase of ovarian androgen in an essentially non ovulatory adult women is presumptive evidence of polycystic ovary syndrome. A high serum FSH to LH ratio (1.9 to 3.8) has been observed in post menopausal women. Hypogonadism is usually associated with increased both FSH and LH levels; while decreased FSH and LH may occur in pituitary or hypothalamic failure.

Table (3) showed high incidence of hirsutism (48%) in working women in batteries plant than in control group (0%). Also percentage of hirsutism in female worker groups increased with increasing exposure time to lead. Some of workers had hirsutism, their serum testosterone levels were high, and this type of hirsutism seems to be androgen induced hirsutism (hyperandrogenism). In this study in spite of decreased serum testosterone levels with prolonged exposure time (fig.1), percentage of hirsutism increased as time of exposure increased (table 3), so it can be concluded that hirsutism may depend on many factors other than androgen (testosterone) levels in female exposed to lead. When hirsutism is associated with obesity and menstrual abnormalities, the source of androgen excess is often ovarian, typically polycystic ovary syndrome. When it is associated with average weight and normal menses, the source is often adrenal and rarely (in <5% of cases) pituitary.

Table (3) showed increased incidence of miscarriages in female worker groups (50%) compared to control group (11%). Lead has toxic action on the trophoblastic epithelium and tonic contraction of the uterus. It therefore results either in abortion or a dead fetus.

In this study when lead exposure time increased, percentage of miscarriages also increased (table 3). Much of the previous literatures focused on an increased incidence of spontaneous abortion and stillbirth associated with lead exposure in the workplace. Other studies have examined the issue of lead’s involvement in spontaneous abortion, stillbirth, preterm delivery, and low birth weight. Women in the studies in Boston, Cleveland, Cincinnati, and Port Pirie had average blood lead concentrations during pregnancy of 5-10 µg/dl; almost all had blood concentration less than 25 µg/dl. In the Cincinnati study, gestational age was reduced about 0.6 weeks for each natural log unit increase in blood lead concentrations. However, the Cincinnati and Port Pirie studies found a lead related decrease in duration of pregnancy, and Cincinnati and Boston studies reported a lead-related decrease in birth weight. In conclusion there are evident changes in sex hormones due to exposure of female workers to lead in Battery manufacturing plant.

References
2. Oliver, P. A lecture on lead poisoning and the race:. Med. ., 1911 : 1096-1098.
Detection of Cholesterol in Suaeda Baccata (Chenopodiaceae)  
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*Department of Pharmacognosy, College of Pharmacy, University of Baghdad, Baghdad-Iraq

Abstract  
This study detects the presence of cholesterol in an Iraqi plant named Suaeda baccata Forsk of the family Chenopodiaceae, wildly and widely grown in Iraq. The absence of any publication concerning the sterol content of this Suaeda specie, and the industrial importance of cholesterol depending on its role as a precursor in the synthesis of some hormones, like progesterone, acquired this study its value. The investigations revealed the presence of cholesterol that was proved by TLC together with the standard compound cholesterol, and anisaldehyde spray reagent using three different solvent systems, then authenticated by HPLC, in which the retention time of both the standard cholesterol and the plant extract cholesterol were identical.

Key Words: Cholesterol, Suaeda Baccat and Chenopodiaceae

Introduction  
The Chenopodiaceae (goosefoot family) is a large family including about 102 genera, and 1400 species of low growing plants. (1) Members of this family including Suaeda baccata mostly grow naturally in soils containing much salt (halophytes) (2).  
Suaeda baccata specie is distributed in Iraq, in south of Jazira District, Southern Desert District, Western Desert Central Alluvial Plain District and Eastern Alluvial District (3), the photo of this plant is demonstrated bellow.  

Literature survey indicated that different species of the genus Suaeda contain several different compounds, including sterols (4, 5), thus it was deemed desirable to find out the sterol content of this plant. Steroids constitute a natural product class of compounds that is widely distributed throughout nature. (6) The chemical structure of steroids is base on (a) perhydrocyclopenta phenanthrene nucleus. The steroidal nucleus is derived from isopentyl pyrophosphate as follows:-

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Received 28-1-2006  
Accepted 4-11-2006
The first steroids isolated from nature were a series of C27 – C29 alcohols that were found in lipid fraction of many tissues. These compounds were solids and therefore named sterols from the Greek *stereos*, meaning solid. The most widely occurring sterol is cholesterol, (Greek Word, chole, meaning bile). It was first isolated from human gallstones. Until recently cholesterol was thought to be restricted to the animal kingdom; however, it has now been identified in plants. \(^{(7, 9, 10)}\) The fact is that the cholesterol widespread in the plant kingdom (although other related sterols, such as β-sitosterol generally occur in higher quantity) \(^{(13, 14)}\). Cholesterol occurs in both free and esterified. It occurs as a component of plant membranes and as part of the surface lipids of leaves where it is sometimes the major sterol. The quantity of cholesterol is generally small when expressed as a percent of total lipid. While cholesterol averages perhaps 50 mg/kg total lipid in plants, it can be as high as 5 g/kg (or more) in animals. \(^{(15)}\).

The quantities of cholesterol in a number of vegetable (plant) oils are given in the following table.

**Table (1) Cholesterol content of some plant oils**

<table>
<thead>
<tr>
<th>Source</th>
<th>Cholesterol (mg/kg)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palm oil</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>Palm kernel</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Coconut oil</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>Cotton seed oil</td>
<td>45</td>
<td>17</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>29</td>
<td>17</td>
</tr>
<tr>
<td>Corn oil</td>
<td>55</td>
<td>17</td>
</tr>
<tr>
<td>Peanut oil</td>
<td>24</td>
<td>17</td>
</tr>
<tr>
<td>Sun flower oil</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>Canola oil</td>
<td>53</td>
<td>17</td>
</tr>
<tr>
<td>Avogadro oil</td>
<td>&lt; 30</td>
<td>18</td>
</tr>
<tr>
<td>Olive oil</td>
<td>0.5-2</td>
<td>19, 20</td>
</tr>
<tr>
<td>Sesame oil</td>
<td>Ca. 1</td>
<td>19, 20</td>
</tr>
</tbody>
</table>

Cholesterol and its esters are important constituents of plant membranes. The following table represents some data on the sterol fraction of some plant organelles.
The proportion of cholesterol in the sterol fraction of Liliaceae, Solanaceae and Scrophulariaceae families is especially large.\(^{(24,25)}\)

Cholesterol (C27H46O), \(3\beta\)-cholesta-5-en-3ol, mol. wt. 386.66, is practically insoluble in water, slightly soluble in alcohol, more soluble in hot alcohol, one gram dissolves in 2.8 ml of ether, in 4.5 ml of chloroform, In 1.5 ml of pyridine, also it is soluble in petroleum ether, benzene\(^{(26)}\). Cholesterol structure is represented below.\(^{(26)}\)

![Cholesterol structure](image)

**Figure (2) cholesterol structure**

Although total synthesis of some medicinal steroids is employed commercially, there is also a great demand for natural products which will serve as a starting material for their partial synthesis\(^{(2)}\).

Accordingly, cholesterol has serve as a precursor for the synthesis of progesterone\(^{(27)}\), as represented in the following diagram, and this acquired this study its importance.

| **Table (2) Sub-cellular distribution of cholesterol in plant** |
|---|---|---|
| **Source** | Free cholesterol % | Cholesterol-ester % | Reference |
| **1. Green bean leaves** |
| a. Whole | 1 | 1 | 21 |
| b. Chloroplasts | 24 | 33 |
| c. Mitochondria | --- | --- |
| d. Microsomes | 1 | 28 |
| **2. Etiolated bean leaves** |
| a. Whole | 6 | 23 |
| b. Chloroplasts | 27 | 26 |
| c. Mitochondria | --- | --- |
| d. Microsomes | 6 | 34 |
| **3. Organelles of 21-day maize shoots** |
| a. Nuclei | 22 | 76 | 22 |
| b. Chloroplasts | 2 | 52 |
| c. Mitochondria | 1 | 32 |
| d. Microsomes | 1 | 32 |

While cholesterol is usually a minor constituent of the sterol fraction in plants, it is the major constituent of some plant surface as shown in table (3)

**Table (3) Sterol content of Rape (Canola)\(^{(23)}\)**

<table>
<thead>
<tr>
<th><strong>Source</strong></th>
<th>Cholesterol %</th>
<th>Sitosterol %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Leaves</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Surface</td>
<td>71.5</td>
<td>0.6</td>
</tr>
<tr>
<td>b. Intracellular</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td><strong>2. Seeds</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Surface</td>
<td>7.2</td>
<td>62</td>
</tr>
<tr>
<td>b. Intracellular</td>
<td>0.7</td>
<td>67</td>
</tr>
<tr>
<td><strong>3. Seedpods</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Surface</td>
<td>35</td>
<td>21</td>
</tr>
</tbody>
</table>
Material and Methods
The plant material (aerial part) was collected during months of July, August, and September 2005. From the high ways of Baghdad city. The plant was identified by the Department of Pharmacognosy, college of Pharmacy/University of Baghdad; and authenticated by the National Herbarium of Iraq, Botany Directorate at Abu-Ghraib, Iraq. Forty grams of the dried aerial parts were first macerated with 500 ml of n-hexane for 24 hours. The residual plant part then was dried at room temperature, soaked in water for 24 hours, dried, and then refluxed with 2 N HCl solution for two hours. After filtration by Buchner funnel, 5% ammonia solution was added to the residual plant part, and then washed by distilled water several times, until neutral. This plant part after drying over night will be extracted again with 250 ml of petroleum ether (60°C - 80°C) for ten hours by the use of soxhlet apparatus. The petroleum ether filtrate will then be evaporated to dryness to be ready for the identification of steroid by TLC. The following diagram represents the extraction procedure of steroids from Suaeda baccata.
Detection of cholesterol in *Suaeda baccata*

40 gm. Of the dried aerial plant part

- Maceration with 500 ml n-hexane For 24hr.

Residual plant part

- 1. Dried at room temp.
- 2. Soak in water for 24hr.
- 3. Reflux with 2NHCl for 2hr.

Filtrate

- Add 5% NH3
- Wash several times with D.W

Plant part

- 1. Dried over night at room temp
- 2. Reflux with petroleum ether (60-800) For 10 hr. (soxhlet).

Marc

Filtrate

- Evaporate to dryness

**TLC detection for steroids**

![Schematic procedure for the extraction of steroids from *Suaeda baccata*](image)

**Identification of the Steroid (Cholesterol)**

Identification was performed first by TLC, using silica gel G, anisaldehyde spray reagent (29,30) 0.5 ml Anisaldehyde is mixed with 10 ml glacial acetic acid, followed by 85 ml methanol and 5 ml concentrated sulphuric acid in that order, the TLC plates are sprayed with 10 ml, heated at 1100 for 5-10 minutes.], standard cholesterol, and different solvent systems that were:

- Solvent (1): Toluene: ethyl acetate (90:10)
- Solvent (2): Benzene: acetone (90:10)
- Solvent (3): Petroleum ether (60-800): ethyl acetate (75:25)

Then this identification was authenticated by HPLC.

**Result and Discussions**

Cholesterol can be found in plant either in the free state or conjugated as simple glycoside (33), therefore the extraction procedure included the use of water and acid, necessary for the cleavage of the glycosidic linkage and the release of the aglycone part (cholesterol), and the possible sugar site attachment is C3- atom in its structure. As the cholesterol is soluble in petroleum ether, therefore this solvent was used in its extraction.

The identification of cholesterol was performed by TLC using three different solvent systems S1, S2, S3. As represented in the following diagrams:-
Detection of cholesterol in *suaeda baccata*

The Rf. value of each solvent system of the standard cholesterol and the plant extract cholesterol, are represented in the following table.

**Table (4) [Rf values of both, the aerial plant part extract and standard cholesterol]**

<table>
<thead>
<tr>
<th>Solvent system</th>
<th>Rf. of standard cholesterol</th>
<th>Rf. of the plant extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1 (Toluene: ethyl acetate (90:10))</td>
<td>0.739</td>
<td>0.730</td>
</tr>
<tr>
<td>S2 (Benzen:acetone (90:10))</td>
<td>0.192</td>
<td>0.192</td>
</tr>
<tr>
<td>S3 (Petroleum ether (60°-80°): ethyl acetate (75:25))</td>
<td>0.513</td>
<td>0.521</td>
</tr>
</tbody>
</table>
Further identification to the cholesterol in the plant extract was performed by HPLC. In which the retention time of both the standard cholesterol and the plant extract cholesterol were identical as represented in the chart below.

**Table (5) : Table of the HPLC conditions**

<table>
<thead>
<tr>
<th>Conditions of cholesterol</th>
<th>HPLC (19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase</td>
<td>Acetonitrile:methanol 50:50 containing 3% D.W</td>
</tr>
<tr>
<td>Column</td>
<td>C18</td>
</tr>
<tr>
<td>Detector</td>
<td>210</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1ml/min</td>
</tr>
<tr>
<td>Injection Volume</td>
<td></td>
</tr>
</tbody>
</table>

**Conclusion**

Suaeda baccata Forsk (Chenopodiaceae), a wild Iraqi plant, contains cholesterol as one of its constituents.

**References**

7. ibid pp. 158,160.
15. Behraman E.J. and Venkat Gopalan "J. of Chemical Education", Ohio State University, Columbus, 2005,Vol.82, No.12.,
Effect of Temperature on the Stability and Release Profile of Ibuprofen Microcapsules

Issraa R. Abed Al-Rahman

Abstract

The stability and releasing profile of 2:1 core: wall ratio ibuprofen microcapsules prepared by aqueous coacervation (gelatin and acacia polymers coat) and an organic coacervation methods (ethyl cellulose and sodium alginate polymers coat) in weight equivalent to 300mg drug, were studied using different storage temperatures 40°C, 50°C, 60°C and refrigerator temperature 4°C in an opened and closed container for three months (releasing profile) and four months (stability study). It was found that, these ibuprofen microcapsules were stable with expiration dates of 4.1 and 3.1 years for aqueous and an organic method respectively. Aqueous prepared ibuprofen microcapsules were found more stable than those microcapsules prepared by organic method with activation energy (Ea) 4804.8 cal/mol and 5033.6 cal/mol of a drug respectively. The releasing percentage of ibuprofen for all microcapsules prepared by both methods was decreased as the storage temperatures increased, except for microcapsules prepared by aqueous method, which were found to be the same at 25-40°C as the standard one which stored at 25°C temperature, on the other hand, as the temperature decreased to 4°C (refrigerator ) of an open and closed container, the amount of drug detected in microparticles is increased. These differences in the amount of drug released may be referred to the change in physical properties in polymer coats or in the amount of drug detected in a whole microcapsules.

Keywords: Ibuprofen Microcapsules, Storage Temperature, Stability.
Introduction:

Microencapsulation technique provides a good tool to protect the drug from environmental factors (temperature, humidity, light, oxygen) by improving its stability through enclosing of small drug particles of liquids, solids or gases by an intact shell. Accelerated stability study at elevated temperatures is an important study which indicates the effect of temperature on chemical stability of microcapsules as well as their physical properties.

Chemically by predicting the rate of drug decomposition after experimentally evaluating the velocity constant of a particular reaction, the amount of active drug present at any time and expiration date could be determined the specific manner in which the rate of reaction varies with the concentration of the reactants.

The Arrhenius equation reveals the effect of temperature on an observed rate constant by affecting the molecular motion through determining the Arrhenius activation energy, which is the minimum kinetic energy that a molecule must possess in order to undergo reaction.

The physical effect of temperature, on microcapsules by altering the glass transition temperature (Tg) of microspheres polymers coat; is a narrow temperature range over which microcapsules polymer reversibly change from phase to phase which occurs in amorphous polymers and in amorphous chains partly crystalline polymers, for example Tg of pure glycolide-L-lactide (PLGA) polymer of microcapsule wall was found to be 49°C at which thermo-reversible gelation in which the long and flexible polymer chains tend to become entangled and attract each other by secondary side chain forces, this physical changes in microcapsules polymer cause an altering in the diffusion of the drug from microcapsules wall by affecting the driving forces of physico-chemical potential gradient and transport parameters.

On the other hand, protein microcapsules stored at 4°C over the course of 28 days produced physical changes in shape or integrity of microcapsules that affected the releasing behavior of these spheres.

Aim of the work

To study the effect of different storage temperatures on the stability (kinetic study) and releasing profile of 2:1 ibuprofen microcapsules prepared by aqueous (gelatin and acacia coat) and organic methods (sodium alginate and ethylcellulose polymer coat).

Materials and Methods


Preparation of Ibuprofen Microcapsules

Ibuprofen microcapsules were prepared freshly using aqueous method (complex coacervation phase separation) and organic method as shown in scheme 1 and 2 respectively.
Accelerated Stability of Ibuprofen Microcapsules

Scheme - 1-
Preparation of ibuprofen microcapsules by aqueous method.

Scheme - 2-
Preparation of ibuprofen microcapsules by organic method.
Assay of Microcapsules content
0.450gm of 2: 1 core : wall ibuprofen microcapsules prepared by aqueous and organic method (eq. to 207 mg (69%) and 261 mg (87%) ibuprofen respectively) was extracted in 50ml of methanol, 0.4ml of phenolphthaline solution as indicator was added, then titrated against 0.1M sodium hydroxide until a red color was obtained, blank titration was carried out, and amount of drug content was determined, each 1ml of 0.1M NaOH is equivalent to 20.63mg of C_{9}H_{10}O_{2}(9,10).

Determination of microcapsules properties
Microencapsulation yield and encapsulation efficiency of 2:1 core : wall ratio ibuprofen microcapsules prepared by aqueous and organic method were estimated using the following expressions.

\[
\text{Actual wt. of microcapsules gained} \times 100 \\
\text{Theoretical wt. of microcapsules}
\]

\[
\text{Actual drug loading} \times 100 \\
\text{Theoretical drug loading}
\]

Effect of temperature on stability of ibuprofen microcapsules:
The effect of different storage temperatures on the degradation rate of the selected formula 2: 1 core: wall ratio ibuprofen microcapsules prepared by aqueous and organic method was studied.
The study was done by incubated the prepared microcapsules in an amber colored glass containers at different temperatures (60°C, 50°C, 40°C) and 4°C of refrigerator in an opened and closed containers for 4 months. Samples of microcapsules of both preparation methods (aqueous and organic) in weight of 652mg and 517mg respectively equivalent to 300mg ibuprofen were taken at desired time intervals (every month) and assayed for the content of drug according to the procedure mentioned before for stability study.

The effect of temperature on the dissolution behavior of ibuprofen microcapsules:
In vitro drug release from 2:1 core: wall ratio ibuprofen microcapsules samples using basket method in weight equivalent to 300 mg drug of both preparation methods was studied using U. S. P dissolution apparatus with 900 ml of pH6.8 phosphate buffer at 37°C ± 1 and stirring speed 150 r.p.m. At appropriate time intervals 5ml sample was withdrawn, filtered, and measured spectrophotometrically at λmax 264nm. This assay was repeated for three months, in order, to study the effect of different storage temperature (40°C, 50°C, 60°C), 4°C opened container and closed one) on the release profile of ibuprofen from selected formula of both methods.

Results and Discussion

Table (1)Microcapsules Contents of 2:1 Core to Wall Ratio Ibuprofen Microcapsules Prepared by Aqueous and Organic Method.

<table>
<thead>
<tr>
<th>(2:1) core : wall ratio</th>
<th>Drug Amount (gm)</th>
<th>Coating (gm)</th>
<th>Microencapsulation yield</th>
<th>Drug loading (mg)</th>
<th>Encapsulation efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gelatin</td>
<td>Acacia</td>
<td>Ethellose - lose</td>
<td>Na-Alginate</td>
</tr>
<tr>
<td>Aqueously prepared ratio</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Organically prepared ratio</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>
Effect of elevated temperature on stability of ibuprofen microcapsules

Figures (1) and (2) show the change in the log % remaining of ibuprofen versus time at different elevated temperature 40°C, 50°C, 60°C. The obtained profiles were linear for both 2:1 core:wall ratio ibuprofen microcapsules prepared by aqueous and organic method indicating that ibuprofen degradation followed first order kinetics.

The slope of this linearity was determined and the calculated rate constants are summarized in tables 2 and 3 for both methods, respectively. Arrhenius plots are shown in figures (3) and (4). The rate constants (K) at 25°C obtained from the plot were 2.13 x 10^{-3} month^{-1} and 2.81 x 10^{-3} month^{-1} for aqueous and organic method respectively.
Table (2): Degradation Rate Constants (K) of Ibuprofen Microcapsules Using 2:1 Core: Wall Ratio at Different Temperatures (Aqueous Method)

<table>
<thead>
<tr>
<th>Temperature</th>
<th>K (Month⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40°C</td>
<td>3.96 x 10⁻³</td>
</tr>
<tr>
<td>50°C</td>
<td>4.75 x 10⁻³</td>
</tr>
<tr>
<td>60°C</td>
<td>6.45 x 10⁻³</td>
</tr>
</tbody>
</table>

Table (3): Degradation Rate Constants (K) of Ibuprofen Microcapsules Using 2:1 Core: Wall Ratio at Different Temperatures (Organic Method)

<table>
<thead>
<tr>
<th>Temperature</th>
<th>K (Month⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40°C</td>
<td>4.67 x 10⁻⁴</td>
</tr>
<tr>
<td>50°C</td>
<td>5.77 x 10⁻⁴</td>
</tr>
<tr>
<td>60°C</td>
<td>7.59 x 10⁻⁴</td>
</tr>
</tbody>
</table>

Since the degradation of the drug followed first order kinetic (straight line), the expiration date could be calculated by the following equation:

$$t_{exp} = \frac{0.104}{K_{25^\circ C}} \text{ (Month)^{-1}}$$

It was equal to 4.1 years (Aqueous) and 3.1 years (Organic).

While the Arrhenius activation energy (Ea) was calculated from the slope of Arrhenius plots. It was found to be 4804.8 cal/mol and 5033.6 cal/mol respectively.

The above results indicated that, the microspheric particles prepared from gelatin and acacia coat (aqueous method) were more stable because of presence of crosslinking agent (formaldehyde) (11), as well as, activation energy was less than that of organic method (12), which is in consistent with the results obtained by Sivakumar-PA who found that the stability of ibuprofen liposomes increased by using a crosslinking agent (13).

The other manifestations of general appearance like color of microcapsules were not changed after 4 months for both formulas.

**Effect of temperature on the dissolution behavior of ibuprofen microcapsules:**

Figures 5 and 6 indicate that no largely difference was appeared in the releasing profile of all stored ibuprofen microcapsules of both methods at 60°C, 50°C, 40°C, 4°C in an opened and closed container (refrigerator) after one month in comparison with standard 2:1 core: wall ratio ibuprofen microspheres prepared by aqueous and organic method stored at room temperature (14). While after second and third months, the releasing behavior of microcapsules for both methods was clearly changed as shown in figures 7, 8 and 9,10 for aqueous and organic method, respectively.

![Figure 5](image)

Figure (5): The releasing behavior of 2:1 core: wall ratio ibuprofen microcapsules prepared by aqueous method stored at different temperatures after one month using 6.8pH of phosphate buffer

![Figure 6](image)

Figure (6): The releasing behavior of 2:1 core: wall ratio ibuprofen microcapsules prepared by organic method stored at different temperatures after one month using 6.8pH of phosphate buffer.
On the other hand, microcapsules stored at 50°C and 60°C provided lower release of drug due to removal of solvent, denser periphery (viscous boundary) of microcapsules, but when the temperature of storage decreased to 4°C of refrigerator of an open and close container, the release of ibuprofen increased because of physically changes in the integrity of microcapsules shapes which became more spherical with smooth surface that provided larger surface area with porous surface after completion of stability study.

Also, microcapsules stored in an open container provided higher release of ibuprofen than closed one due to presence of humidity in the refrigerator environment that made the gelatin swell faster in the dissolution media with rapid diffusion of the drug from the texture of the gel.

The results obtained from figure 9 and 10 showed the delay in releasing profile of ibuprofen from microcapsules prepared by organic method when the temperature of storage increased from 40°C to 60°C due to tendency of microcapsules to sticking which was reversible after cooling to room temperature. The slightly decreased of dissolution rate and the sticking phenomena could be explained by change in the polymer film due to its low glass transition temperature, which could be additionally reduced by dissolved ibuprofen molecules in addition, storage at higher temperature allowed ethyl cellulose film to become more dense due to curing effect as it was reported for eudragit L100-55 polymer.

In spite of increasing the release of ibuprofen from microcapsules prepared by organic method when stored at refrigerator temperature in comparison with standard 2:1 core: wall ratio microcapsules prepared by aqueous method stored at 4°C in which the release of the drug was the same as the standard one.

The same result was obtained by Carol-AS, he found that the release of tryptophan and theophylline from gel microcapsules stored at 25°C and 40°C remained unchanged.

Figures 7 and 8, illustrate the releasing behavior of 2:1 core: wall ratio ibuprofen microcapsules prepared by aqueous method stored at 40°C in which the release of the drug was the same as the standard one.

The same result was obtained by Carol-AS, he found that the release of tryptophan and theophylline from gel microcapsules stored at 25°C and 40°C remained unchanged.
Conclusion

2:1 core: wall ratio ibuprofen microcapsules prepared by aqueous and organic method were stable with shelf life of 4.1 and 3.1 years. All physical changes affected the releasing behavior of this ratio occurred at different storage temperature were reversible on leaving these formulas at 25°C in tightly closed container.

References

3. Ismat A. Hamid, Industrial pharmacy, 1975; 201-204.
Evaluation of Analgesic Activity of Newly Synthesized Phthalyl-tyrosyl-glycin Sodium

Muthanna S. Al-Taee*, Kawkab Y. Saour*, Haider M. Mohammed* and Kawkab Y. Saour**

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** Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad – Iraq.

Abstract

Alteration in the backbone structure of the endogenously released opioid peptides Leu⁵/Met⁶ enkephalins may result in compounds having comparable profile of pharmacological activity but with different physicochemical properties and side effects. Phthalyl amino acid and phthalyl esters are among the derivatives that have been synthesized and evaluated for their antibacterial and antifungal activities. This study was conducted to evaluate the possible analgesic activity of phthalyl-tyrosyl-glycin sodium that has been recently synthesized by our team. The study was carried out on 24 albino mice using hot plate method. The animals were allocated into three groups; the first group received saline and represent a control group; the second group received morphine HCl as a standard drug; and the third group received phthalyl-tyrosyl-glycin sodium. The onset with which the animal lift his forearm and the number of jumps per 25 seconds were recorded for each group.

The results of this study showed that phthalyl-tyrosyl-glycin sodium resulted in significant improvement (P<0.05) in analgesia score as well as significant delay in the onset of induced hyperalgesia in comparison to saline-treated group, and in comparison to morphine HCl, no significant difference (P>0.05) was observed in analgesia score but with significant delay in induced hyperalgesia. The results obtained in this study provide experimental evidences for the effectiveness of the prepared compound as analgesic with comparable effect to that of morphine.

Key words: Phthalyl-tyrosyl-glycin sodium, phthalyl group, analgesia.

اكشافات

إن بعض التغييرات الكيميائية على الببتيدات الحياتية المورفية الداخلية (ليوسين وميثيونين) قد تتكون من مواد مكملة للكيانات الدوائية المقاومة ل痛み، ولكن بعضها كيميائيًا، ويتغير مع التفرد في بعض الأعراض الحياتية ذاتية، وتستعمل تعتبر المركبات الحيوية على الفولاذ التقاسيم الأصائل واستر الفولاذ من المركبات المستخرجة والمحمولة، فإن الأطعمة الجرثومية والفيطرية، وعندئذ يفتح هذه الدراسة لقياس الفعالية الدوائية لمركب (Phthalyl-tyrosyl glycine sodium) تتم توزيع الحيوانات على ثلاثة مجموعات، حيث تتم المجموعة الأولى بالسائل الماء (saline) وتم التحكم في المجموعة الدقيقة والمجموعة الثانية بالمركب (Phthalyl-tyrosyl glycine sodium) الذي هو موضوع البحث. تم احتساب الوقت المستغرق لتسحب الحيوان قدمه عن الضفيرة الباردة بعد الفترات، وتم تلقيح الفئات بالفراغ في الفترات خلال 25 دقيقة أظهرت النتائج أن هناك تحسن واضح وفعال في الوقت المستغرق لتسحب الحيوان قدمه عن الضفيرة وذلك في عد الفترات خلال 25 دقيقة في الحيوانات المعالجة بالمركب الجديد غير ملحوظ في معالفة الضفيرة. لذا فريق معول في معدل فور نسب ضرب الحيوانات المعالجة بالمركب الجديد بالمقارنة مع مجموعات الضفيرة الناقصة، إلا أن سرعه نسب ضرب الحيوان قدمه عن الضفيرة الباردة كان (glycin-Phthalyl-tyrosyl) المركب متمكن لâtى قوي ويستعمل بشكل مقارنة للمورفين.

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Received 24-7-2006
Accepted 20-12-2006
Introduction

Opioid peptides, defined as peptides with opiate-like pharmacological effects, are the oldest pharmacological substances known for their analgesic, euphoric and addictive effects. Morphine is the first narcotic analgesic alkaloid isolated from plant source in 1806. In 1975, Hughes and Kosterlitz succeeded in isolating two pentapeptides, Leucine- and Methionine-enkephalin (Leu/Met enkephalin) from pig brain, which compete strongly with morphine-like drugs for binding to receptors in the brain with pharmacological actions resembling those of morphine itself. However, to date, morphine-like compounds remain the only class known to act by mimicking these peptides. Several families of peptides were discovered with multiple categories of opioid receptors.

Binding studies by Synder and colleagues (1973), demonstrated that opioids are recognized by specific receptors. Various pharmacological observations like analgesia, sedation, antitussive, anti-diarrheal, pupillary constriction, and bradycardia produced by different drugs implied the mediation of more than one type of receptors.

Receptor cloning studies revealed the existence of six different opiate receptors named as μ (mu), δ (delta), κ (kappa), σ (sigma), ε (epsilon) and NFQ (nociceptin FQ). These receptors mediate several pharmacological and side effects of opiates including: analgesia (supraspinal, spinal, peripheral), respiratory depression, reduced GI motility, euphoria, dysphoria, sedation and physical dependence.

Concerning their molecular pharmacology, these opioid receptors belong to the family of inhibitory G-protein coupled receptor, mediate the reduction of intracellular cAMP level as a main event beyond receptor binding. Other mechanisms involve the opening of K⁺ and block of Ca²⁺-channels thereby reducing both, neuronal excitability and transmitter release.

Understanding the powerful molecular, biological and physiological terms of opioids was used to develop analgesic compounds with significant advantages over morphine. In this respect, incorporation of a new group in the main backbone of naturally occurring enkephalin was aimed in synthesizing compounds with possible analgesic effects. On the other hand, one of the earliest types of structural modifications applied to the enkephalin was shortening of the peptide chain by removal of residues from the essential sequence or by removal of residues from C-terminal. These experiments showed that significant potency remained in producing analgesia in vivo as in Tyr-D-ala-phe-met amide.

In previous work, Muthanna, et al. (2005) were succeed in designing and synthesis of enkephalin analogues having even shortest chain by removing the C-terminal residue glyphe-leu/met; keeping N-tyrosine residue protected by phthalyl group. Accordingly, the newly synthesized phthalyl-tyrosyl-glycin and its sodium salt (Fig. 1 and 2) are novel compounds produced in our laboratories with possible analgesic activity. The present study was conducted to investigate the analgesic effect of the sodium salt of this compound on experimental animals.

![Figure 1](Phthalyl-tyrosyl-glycin.png)

![Figure 2](Phthalyl-tyrosyl-glycin-sodium-salt.png)

Material and Methods

**Animals:** Twenty-four adult male albino mice weighing 25.23 ± 3.1 gm were used in this study. They were obtained from Iraqi Sera and Vaccine Institute and were housed under standard conditions in the animal house of the College of Pharmacy-University of Baghdad. Animals were fed commercial pellet and tap water in free access ad libitum.

**Materials:** Morphine HCl was supplied by May and Baker Ltd, England. Phthalyl-tyrosyl-glycin has been obtained from reference and its corresponding sodium salt has been synthesized according to reference (14) to increase its water solubility to be suitable for intraperitoneal administration. All compounds
were dissolved in normal saline and were administered intra-peritoneally.

**Methods:** Hot plate method as described by Woolfe and MacDonald was used for evaluation of the analgesic effect of the tested compound compared with morphine as a reference. Animals were allocated into three groups: first group received normal saline, second group received morphine HCl (May and Baker Ltd, England), and third group received phthalyl-N-tyrosyl-glycin sodium. The plate was heated to 55°C and the animal was put on the plate. The onset with which the animal lift his forearm and the number of jumps per 25 seconds were recorded for each group. The results were expressed as mean ± standard error and were analyzed using ANOVA and unpaired Student t-test.

**Results and Discussion**

The data presented in table (1) clearly showed that animals in control group lift their forearms in about (1.2 ± 0.66) seconds which represents the normal onset of heat-induced hyperalgesia. The animal jumps 24.8 ± 2.4 times/25 seconds which represents the analgesia score. Table (1) also showed that morphine significantly (P<0.05) delayed the onset of heat-induced hyperalgesia (2.6 ± 0.74) in comparison to control group and significantly (P<0.05) improved the analgesia score (11.0 ± 2.4) in comparison to control group (24.8 ± 2.4) (Fig. 3 and 4). Interestingly, phthalyl-tyrosyl-glycin sodium resulted in significant (P<0.05) improvement in analgesia score (10.8 ± 1.43), an effect seems comparable to that of morphine (11.0 ± 2.4, P<0.05). However, phthalyl-tyrosyl-glycin sodium showed slightly more significant delay in the onset of heat-induced hyperalgesia over that of morphine (Fig. 3 and 4).

The pharmacologic results may indicate that tyrosine is the essential amino acid residue within the peptide chain of leu / met enkephalin; and the enzymatic resistance is doubtless an important factor in the high potency of this analgesic because, chemically, the presence of phthalyl group may enhance the availability and hence the binding of the compound to opioid receptors and this in turn may potentiate the pharmacological effect of the tested compound, this may explain why the compound showed a comparable analgesic effect to that of morphine. Furthermore, the bulky phthalyl group may increase the lipophilicity and thus enhance a sufficient bioavailability and tissue penetration, an effect which may explain why the compound has slightly faster onset of action than morphine. On the other hand, the presence of bulky phthalyl group is suspected to reduce enzyme degradation of phthalyl-tyrosyl-glycin inside the body by adding a steric hindrance; however, this expectation requires further pharmacokinetic studies.

In conclusion, phthalyl-tyrosyl-glycin sodium showed promising analgesic activity approximately equal to that of morphine with reliable onset of action, making it a good candidate for further development in the field of morphine-replacement therapy based on the idea that enkephalins are compounds devoid of addictive liability.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Jumps/25 seconds</th>
<th>Onset of hyperalgesia (sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (n=6)</td>
<td>24.8 ± 2.4</td>
<td>1.2 ± 0.66</td>
</tr>
<tr>
<td>Morphine-treated group (n=6)</td>
<td>11.0 ± 2.4</td>
<td>2.6 ± 0.74</td>
</tr>
<tr>
<td>Phthalyl-tyrosyl-glycin sodium-treated group (n=6)</td>
<td>10.8 ± 1.43</td>
<td>4.2 ± 1.04</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM. Values with non-identical superscripts (a, b, c) among different groups are considered significantly different (P<0.05).

![Figure 3](image-url): The effect of phthalyl-tyrosyl-glycin sodium and morphine on analgesia score in mice recorded 25 seconds. Results are expressed as the mean (jumps) ± SEM; n=8; non-identical superscripts (a, b) represent significant differences among groups by ANOVA (P<0.05)
References


