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Purification and Characterization of Protease extracted from *Bacillus licheniformis* (B1)

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Abstract

Protease was purified by two steps included precipitation with 70% saturation ammonium sulphate and ion exchange chromatography by (DEAE–Sephadex). The enzyme specific activity was 86.6 U/mg. and fold of purification was 2.72 with 45 % enzyme recovery. Characterization of enzyme showed that the optimum pH for enzyme activity and stability was 9.0 (86 U/ml). Maximum enzyme activity appeared at 50°C. (88U/ml.). The enzyme activity remained was 87% at 50°C for 30 min. Effect of some metal ions, reducing agents and chelating on purified protease was studied, the remaining activity was 110% when it incubated with 10 mM of Mn2+, whereas the remaining activity was 95% and 92% when enzyme incubated with 10 mM of Ca2+ and Mg2+, respectively. There was little effect for 10 mM of PMSF on enzyme activity, whereas the activity declined to 18% when enzyme was treated with 10 mM of 10 mM EDTA .

Keywords: Protease, Purification, Characterization, Bacillus licheniformis.

تنقية وخصائص انزيم البروتييز من بكتريا Bacillus licheniformis B1

حيدر جواد كاظم وسناء برهان الدين

قسم علوم الحياة، جامعة بغداد، بغداد، العراق

الخلاصة :

نقي الانزيم بخطوتين تضمنت الترسيب بكبريتات الامونيوم بنسبة تشبع 70% ثم كروموتوغرافيا التبادل الايوني بأستخدام عمود DEAE-Sephadex ،وكانت الفعالية النوعية للانزيم المنقى (.86.6U/ml) و بعدد مرات تتقية 2.72 وبحصيلة انزيمية مقدارها 45%. تمت دراسة توصيف الانزيم المنقى، وأظهرت النتائج بان افضل فعالية انزيمية وثباتية كانت عند الرقم الهيدروجيني 9 (U/ml88)، واظهرت اقصى فعالية للانزيم عند درجة حرارة 50.م° (80.ml88) واحتفظ الانزيم بـ(87%) من فعاليته عند نفس درجة الحرارة لمدة 30 دقيقة. درس تأثير بعض الايونات الفلزية والمواد المختزلة والكلابية على فعالية الانزيم المنقى، ولوحظ زيادة في في فعالية الانزيم بحدود 110% عند حضنه مع ايون المختزلة والكلابية على فعالية الانزيم المنقى، ولوحظ زيادة في في فعالية الانزيم بحدود 100% عند حضنه مع ايون بعد 200% و 92% على التوالي، كما لوحظ تأثير قليل لمادة 2019 بتركيز من ايوني هـ2019 النسبة المؤية للفعالية الى 18% عند معاملة الانزيم بمادة 200% مع نفس التركيز من ايوني معاد النسبة المؤية للفعالية الى 18% عند معاملة الانزيم بمادة 200% مع نفس التركيز من ايوني 100% عند معاديم الخضات الفريس المنترية المؤية المولار بينما كانت الفعالية المتبقية للانزيم عند حضنه مع نفس التركيز من ايوني هـ2014 المؤلنت النسبة المؤية للفعالية الى 18% عند معاملة الانزيم بمادة 200% بتركيز 10 ملي مولار في حين انخفضت

الكلمات المفتاحية: انزيم بروتييز ، تتقية، توصيف، Bacillus licheniformis

INTRODUCTION

Proteases or Peptidases are enzymes that catalyze hydrolytic reactions (hydrolysis peptide bond) in which protein molecules were degraded to peptides and amino acids. According to the Commission (EC) Enzyme classification, proteases belong to group 3 (hydrolases), and subgroup 4 (which hydrolyse peptide bonds) (1). They are necessary for the survival of all living creatures, and they are encoded by about 2% of genes in all kinds of organisms (2). They are important for many biological processes and the versatility of proteases ranging from being the major armour of protein degrading saprophytes to the signal sequence cleaving peptidase enzymes of higher organisms, clearly illustrates their influence in the biosphere (3).Proteases have found a wide range of applications in various industries such as food, pharmaceutical, cosmetic, etc. and have been widely commercialized by various companies throughout the world (4). The metabolic diversity of Bacillus licheniformis has led to its exploitation in a variety of bio-industrial processes, including production of enzymes (5). It is considered as one of the examples of thermophilic bacteria belongs to Bacillus genus (6). Its optimal growth temperature is around 50-55°C (7). Researches interested in these has organisms increased due to their biotechnological potential, especially as sources of thermostable enzymes (8). The advantage of using enzymes from thermophilic microorganisms is that the thermostability of such proteins at high temperatures is an intrinsic property (9). The alkaline proteases resulting mainly from Bacillus licheniformis were used in detergents and occupy a large portion of the market (10). This study was aimed to isolate locally highest protease producer Bacillus and purified to used in many applications and economical advantages

MATERIALS AND METHODS

All experiments achieved in duplicate.

Extraction of enzyme

B.licheniformis cultured under optimum conditions composed (soluble casein 0.5 g, yeast extract 0.5g, glucose 0.1g, KH_2PO_4 0.02g, K_2HPO_4 0.02g, $MgSO_4.7H_2O$ 0.01g and D.W, and incubated for 48h. at 37 °C (11). The enzyme was extracted by extracted by cooling centrifuge at 3000rpm for 45min, the enzyme activity and protein concentration were assayed in the supernatant (crude enzyme) and specific activity was measured.

Assay of Protease Activity

Protease activity determined was spectrophotometrically according to method of Anson (12) with some modification. Enzyme extract solution 0.2 ml was incubated with 1.8 ml casein solution at 40°C for 15min. The blank, consisted of 1.8 ml casein solution and 3.0ml 5% TCA (trichloroacetic acid) and 0.2 enzyme solution. The reaction was stopped by the addition of 3.0ml 5 % trichloroacetic acid and incubated at 25°C for 10 min. The mixture was centrifuged for 10min. then supernatant was separated. Quantity 2.5ml of 0.5M Na₂CO₃ solution was added to 1 ml of the supernatant, and 1ml Folin-Ciocalteus reagent was added and Incubated at 37°C for 20 min. The absorbance (O.D.) was measured at 600 nm for solution. One unit of protease activity was defined as the amount of enzyme required to liberate one µg tyrosine per minute per ml under assay conditions.

Purification of enzyme

1. Precipitation of enzyme with ammonium sulfate

The crude protease solution was precipitated with different concentration of ammonium sulfate (40% - 80%) saturation under cooling condition, the precipitates were separated by centrifugation at 3000 rpm for 45 min and dissolved in small amount of buffer 0.2M Tris- HCl. The final volume of solution, activity of enzyme and protein concentration were measured and specific activity was calculated.

2. Dialysis of enzyme

The precipitated protein solution was dialyzed against 0.2M of Tris- HCl buffer over night at 4°C. The dialyzed enzyme was concentrated with sucrose and kept at 4°C for the next step of purification.

3. Ion exchange chromatography by DEAE-Sephadex

The enzyme solution (3ml.) was loaded on DEAE-Sephadex column (2.5x20cm). The column was washed with 0.2M Tris-HCl buffer pH 8.0 and eluted with gradient (0.1-03M) NaCl solution at flow rate of 30ml/h. Fractions of 5ml./tube were collected and the optical density at 280nm. was measured. The fractions with high protease activity were collected, volume, enzyme activity and protein concentration were estimated..

Characterization of purified protease.

Determination of optimum temperature for protease activity.

Quantity 1.8 ml of reaction solution was incubated at different temperature (30, 37, 40, 50, 60 and 70 °C) 0.2 ml of enzyme was added to reaction solution at each temperature and incubated for 15 min and then the enzyme activity was assayed.

Determination of protease stability at different temperatures.

One ml of purified protease was incubated in water bath at 30, 40, 50, 60, 70, 80, 90, and 100°C for 30min, and immediately transferred into an ice bath. The enzymatic activity was measured and the remaining activity was calculated percentage and plotted against the temperature. The remaining activity was estimated according to the following equation:

Activity of enzyme after treatment Remaining activity (%) = ______ ×1... Activity of enzyme before treatment

4

Determination of optimum pH of protease activity.

Reaction solution was prepared at different pH values (6.0-11.0). 0.2 ml enzyme solution was mixed with 1.8 ml reaction solution and incubated at 50 °C for 15 min then enzyme activity was assayed.

Determination of protease stability at different pHs.

Equal volumes of purified enzyme and buffer solutions with pH range (6 to 11) were incubated at a 25°C for 30 min. and cooled in ice bath. The enzymatic activity for each treatment was measured and the remaining activity (%) for protease was calculated.

Determination of metal ions and inhibitor effects on protease activity.

One ml. of purified enzyme was mixed with 1ml of 20 mMmetal ions solution CaCl₂, MnCl₂

and MgCl₂. EDTA solution and PMSF, then incubated at 25°C. for 30 min. The final concentration of each one was 10 mM 0.2ml. of each enzyme solution mentioned earlier was mixed with 1.8ml. of reaction solution and incubated at 50°C for 15min. Then enzyme activity was assayed and remaining activity was calculated.

Statistical Analysis.

The Statistical Analysis System- SAS (13) was used to determine the significant differences between the different parameters. LSD test (Least Significant Difference). $P \le 0.05$ was applied to the compare between means.

RESULTS AND DISCUSSION

Purification of protease.

Ammonium sulphate precipitation was achieved using different percentages of saturation ratio

ranging between (40% -80%) to concentrate the protease produced by *B. licheniformis*B1. Result in Figure (1) showed that specific activity of protease increased gradually with increasing of saturated percentage up to (10.77U/mg) at 70% concentration. Fold of purification was 1.76 with 52.9% recovery. Salting out using ammonium sulfate is one of the classical methods in protein biochemistry. Formerly it was widely used for the fractionation of proteins, it rather used as an inexpensive way of concentrating a protein extract (14).

Ammonium sulfate is favored in precipitation step due to its high solubility, availability, being cheap and it does not damage most enzymes (15).Ahmed, *et al.*, (16) isolated protease from *Bacillus subtilis* and precipitated at 70% saturation with specific activity of 55.71 U/mg and 1.11 fold purification.

Enzyme solution was applied to DEAE- Sephadex column and washed with 0.2M trisHCl buffer pH8.0 to remove uncharged and positively charged proteins in enzyme solution. The bound proteins were eluted using gradient concentration of (0.1-0.3) M. sodium chloride. The result showed two protein peaks in the eluted fractions, only the first peak represented protease, located at fractions 11-17 which eluted with 0.2M sodium chloride solution (Figure 2). The result indicated that protease has a negative net charge since it bounds with anionic ion-exchange. The specific activity increased in this step to 16.6 U/mg proteins, with 2.72 fold of purification and 45% recovery (Table 1). Chromatography has become an essential tool in biochemestry laboratory as, protein purification is widlyneeded (17). Ion exchange chromatography is prefer in protein purification, it can distinguish between two proteins different with one amino acid (18). This technique uses materials such as Sephadex or cellulose which have high capacity for bioseparation, easy to prepare, multiple use, in addition to simplicity to separate different biomolecules, principle which depending on charge difference (19). Akel, et al., (20) used DEAE-Sepharose as a final step for purification of thermostable protease from Bacillus strain

HUTBS71, with 59 fold of purification and 1.7% recovery. Banik and Prakash, (21) purified laundry detergent compatible alkaline protease from *Bacillus cereus* by anion exchange chromatography with 5.32 fold of purification and 53.61% recovery.

Characterization of purified protease

Effect of temperature on protease activity.

Protease activity was assayed at different temperatures ranging from 30°C to70°C (Figure 3). The results showed that enzyme activity increases with temperature increasing within the range of 30 °Cup to 50 °C the maximum enzyme activity (88u/ml) at 50 °C, a reduction in enzyme activity was observed above 50°C. The decrease in enzyme activity at high temperatures due to the destruction of enzyme or changes in its tertiary structure (22). The lowoptimal temperature for protease activities is desirable for detergent formulations for washing at normal temperatures (23). Similar reports were obtained for Bacillus sp. MIG (24) and Bacillus sp. CEMB10370 (25). Sangeetha, et al. (26) reported that protease from B. licheniformis VSG1 exhibited maximum activity at 45°C. While, alkaline protease from B. mojavensis was optimally active at 60°C.with rapid loss of activity above 65°C (27). However, this result gives the protease an economic property since it can be used as

catalyst in a wide range of temperature.

Effect of temperature on protease stability.

The thermostability of the protease was examined. The results indicated that the enzyme was nearly stable at a temperature 30, 40 and 50°C for 30min. the remaining activity was 95%, 95% and 87% respectively, while a significant reduction in enzyme stability was observed above 50°C.(Figure4). This indicating that *B. licheniformis* B1 protease is moderately stable at hightemperature and hence it remains active at temperature above the normal or physiological

temperature, this property make the enzyme at high temperature. Relative activity of protease enzyme produced by *B.clausii* was reported to be 100% after incubation the enzyme at temperature ranging from 30° C to 65° C for 60 min. (28). Akel, *et al.* (20) found that protease from *Bacillus* strain HUTBS71was stable after incubation at 50°C. and 60° C. for 2h. and retained 84% of its original activity.

Effect of pH on protease activity

The pH range from 6.0 to 11.0 was used to study the effect of pH purified protease activity (Figure 5). The results showed that protease is active in a wide range of pH (6.0-11.0) but it is more active at pH 9.0 than other value. The activity was (86U/ml.). The pH effect on enzyme activity in different ways; on the ionization of groups in the enzyme's active site, on the ionization of groups of substrate, or by affecting the conformation of either the enzyme or the substrate (14), which could explain the decrease in the activity value of pH 6.0 and 11.0.The present result was in line with the findings obtained for the optimum pH for enzymatic activity of other Bacillus species: for B. cereus KCTC 3674 (29). Olajuvigbe and Ajele.(30) reported protease from *B.licheniformis* optimum Lbbl-11 shows the pН 8.0. However, different finding reported by Chantawannakulet al. (31) who isolated Bacillus from fermented soybean they found the optimum pH for protease was 6.

Effect of pH on protease stability.

The purified *B. licheniformis*B1 protease was incubated in different pH (6.0-11.0) for 30min, the results showed that protease has good stability in pHs 8 - 10 with highest remaining activity at pH 9 (83%) (Figure 6). The activity decreased slightly at pHs 10 and 11 the remaining activity were 71% and 61% respectively, whereas more than half of enzyme activity lost at pH6. This result may give a conclusion that the protease of *B. licheniformis*B1 is more stable in alkaline pH than neutral and acidic pH. The effect of pH on the enzyme stability could be explained in the formation of improper ionic form of enzyme or the active sites and irreversible inactivation (32). Generally, most of the commercial available alkaline proteases are active in the pH and temperature range between 9.0–12.0 and 50– 60° C, respectively (27). The effect of pH on stability of alkaline protease from *B.licheniformis* LBBL-11 showed that the enzyme had optimum pH for stability at 7.0 it retained 86% of its activity at pH 11.0 (30).

Metal ions and inhibitor effects on protease activity.

Results mentioned in table (2) showed that the protease activity increased when incubated with 10mM. Mn^{2+} while Ca^{2+} and Mg^{2+} slightly decreased protease activity at the same concentration. From these results it can be concluded Mn²⁺ was the most effective ion since it increase the remaining activity to (110%) Ca^{2+} and Mg^{2+} nearly have no effect on enzyme they gave 95% and 92% respectively. Suggesting that metal ions had a capability to protect enzyme denaturation.Where, against Akelet al.. (20).mentioned that these metal ions protected the enzyme from thermal denaturation and maintained its active conformation at the high temperature. Some protease required a divalent cation like Ca⁺² and Mn^{+2} or combination of these cations for its maximum activity (33).

Furthermore, these cations may enhance the stability of a *Bacillus* protease (34). Shaheen*et al.*, (22) found that strong inhibition or stimulation by metal ions in case of protease activity, was not observed. The results revealed a slight decrease in protease activity occurred after incubation with 10mM. phenyl methyl sulfonylfloride, while it lost most of its activity after incubation with 10mM. EDTA (chelating agent). This mean that the protease produced by *B. licheniformis* B1 is

not serine protease but it is metallo-protease which require metal ions for its activity removal of metal ions from enzyme structure leads to entire loss enzyme activity (35). Bacillus are known to secrete two major types of proteases, and metallo-protease, both having serine application in industries (25). The results of Akelet al., (20) in their study on protease from Bacillus strain HUTBS71 indicated that the presence of 1mM EDTA had slight inhibitory effect on protease activity. According to the report of Arulmaniet al. (4) EDTA mild inhibitory effect was observed on serine protease from thermostablealkalophilic Bacillus laterosporus-AK1.

CONCLUSION

Ammonium sulphate at 70% saturation was used to precipitated the protease enzyme prodused from *B. licheniformis*B1. Enzyme was purified by Ion exchange chromatography by DEAE-Sephadex. The net charge of enzyme was negative. The enzyme was active with broad rang of pH an temperature. This enzyme mostly, metalloprotease and Mn^{+2} enhance its activity.



Figure 1: Specific activity of *B. licheniformis* B1 protease after precipitation with ammonium sulphate.



Figure2: Purification of protease produced by *B. licheniformis* (B1) using ion exchange chromatography DEAE-Sephadex column (2.5x20)cm. the fraction were collected with 5ml/tube at flow rate 30ml/hr and eluted with (0.1-0.3)M NaCl solution.

Steps	Volume	Enzyme	Protein	Specific	Total	Fold of	Yield
	(ml)	Activity	conc.	activity	activity	purification	%
		(U/ml)	(mg/ml)	(U/mg)	(U)		
Crude	80	43.3	7.1	6.1	3464	1	100
70% (NH4) ₂ SO ₄ precipitation	25	73.3	6.8	10.77	1832	1.76	52.9
Ion- exchange DEAE .Sephadex	18	86.6	5.2	16.6	1559	2.72	45



Figure 3: Effect of temperature on purified *B. licheniformis* B1 protease activity. [LSD Value: 0.05 = 12.64]



Figure 4: Effect of temperature on purified *B. licheniformis* B1 protease stability. [LSD Value: 0.05 = 13.75]



Figure 5: Effect of pH on purified *B. licheniformis* B1 protease activity. [LSD Value: 0.05 = 8.592]

Figure 6: Effect of pH on stability of purified *B. licheniformis* **B1 protease.** [LSD Value: 0.05 = 6.334]

DY B. achengormis BI.		
Reagent	Concentration (mM)	Remaining activity (%)
CaCl ₂	10	95
MnCl ₂	10	110
MgCl ₂	10	92
PMSF	10	78
EDTA	10	18
Control		100
LSD Value : 0.05		5.292 *

 Table (2): Effect of metal ions and inhibitors on protease activity produced

 by B. licheniformis B1

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Effect of Metformin on Adenosine Deaminase Activity in Polycystic Ovarian Syndrome patients

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Abstract

This study was done to assess the role of adenosine deaminase (ADA) in women with polycystic ovarian syndrome (PCOS) on metformin therapy. The present study is a cross-sectional study (2011/2012) at Al-yarmouk teaching hospital. A total of 80 patients with PCOS were involved in this study, they were classified as newly diagnosed women with PCOS G1: (n=40);women with PCOS on metformin therapy 500 mg (bid) for 90 days G2: (n=40). A matching group of forty apparently healthy women who were included as controls(n=40). Serum ADA was measured in all women and it was significantly reduced in women with PCOS receiving metformin therapy when compared with newly diagnosed patients with PC (p < 0.001) also a significant reduction was found when controls compared with the newly diagnosed women (p < 0.001); however, the reduction was insignificant when controls compared with treated group (p < 0.05). In conclusion_athe results of this study suggest that the metformin currently used by PCOS patients has indirect effect on ADA activity through improving insulin sensitivity.

Keywords: adenosine diaminase, PCOS, metformin.

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تأثير عقار المتفورمين على فعالية أنزيم الأدينوسين دي أمنيدز لدى مريضات متلازمة تكيس المبيض المتعدد

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الخلاصة الهدف من هذه الدراسة هو لتقبيم منزلة انزيم الأدينوسين دي امينيز في متلازمة تكيس المبيض المتعدد. إنّ الدراسة الحالية هي دراسة مقطعية-عرضية للفترة (2011–2012) في مستشفى اليرموك التعليمي، اشترك في هذة الدراسة 80 مريضة بمتلازمة تكيس المبيض المتعدد، تم تقسيمهم كالآتي: 0 مريضات مصابات بمتلازمة تكيس المبيض المتعدد المشخصين حديثا ويدون علاج ج1 (العدد =40) م مريضات مصابات بمتلازمة تكيس المبيض المتعدد وعلى علاج بالمتقورمين بجرعة 500 ملغم مرتين في اليوم 0 مريضات مصابات بمتلازمة تكيس المبيض المتعدد وعلى علاج بالمتقورمين بجرعة 500 ملغم مرتين في اليوم 1 مدة 90 يوم ج2 (العدد =40) اضافة الى مجموعة مقارنة من نساء اصحاء ظاهريا تم اعتمادهم كمجموعة سيطرة ج 3(العدد = 40). تم قياس تركيز انزيم الادينوسين دي امينيز لكل النساء واظهرت النتائج انخفاض معنوي في تركيز انزيم الادينوسين 1 منافة الى مجموعة مقارنة من نساء اصحاء ظاهريا تم اعتمادهم كمجموعة سيطرة ج 3(العدد = 40). دي امينيز في مصل الدم في المريضات مصابات بمتلازمة تكيس المبيض المتعدد وعلى علاج بالمتقورمين بجرعة 500 ملغم مرتين باليوم لمدة 90 يوم ج2 كما هو مقارن بالمريضات المصابات بمتلازمة تكيس المبيض المتعدد 1 المشخصات حديثا ويدون علاج ج1 ولم يسجل اي فرق معنوي بالمقارنة مع مجموعة السيطرة (90.00) على 1 الرغم من وجود ارتفاع معنوي في ج 1 بالمقارنة مع السيطرة (100.00 م). أظهرت النتائج ان عقار المتقورمين 1 الرغم من وجود ارتفاع معنوي في ج 1 بالمقارنة مع السيطرة (100.00 م). أظهرين النتائج ان عقار المتقورمين 1 المتعم مقاومة الإنسولين بزيادة حساسية الإنسجاد له تاثير مباشر على انزيم الادينوسين دي امنيزمن

الكلمات المفتاحية: الادينوسين دي أمينيز ، المتفورمين، متلازمة تكيس المبيض المتعدد.

Introduction

Polycystic Ovarian Syndrome [PCOS] is a relatively common endocrine disorder in women of reproductive age group. It is found in around 70% of women who have ovulation difficulties leading to sub-fertility [1].

PCOS is a condition that has cysts on the ovaries that prevent the ovaries from performing normally. Symptoms of PCOS include Amenorrhea or infrequent menstruation, irregular bleeding, infrequent or no ovulation, multiple immature follicles, increased levels of male hormones, male pattern baldness or thinning hair, excess facial and body hair growth, acne, oily skin or dandruff, dark colored patches of skin specially on neck, groin, underarms, chronic pelvic pain, increased weight or obesity, diabetes, lipid abnormalities and high blood pressure [1].

Fertility problems experienced by women with PCOS may be related to the elevated hormone, insulin or glucose levels, all of which can interfere with implantation as well as development of the embryo [1]. Increased Leutenizing hormone reduces the chance of conception and increase miscarriage. Additionally abnormal insulin levels may also contribute to poor egg quality, making conception more difficult [1]. Insulin resistance (IR) is known to play a critical role in the pathophysiology of PCOS [2]. The administration of insulin sensitizer metformin (MET) is recognized as a successful treatment for many metabolic and reproductive dysregulations characteristic of women with PCOS [3].

Adenosine DeAminase[ADA] is an enzyme that converts adenosine into inosine through an irreversible deamination reaction [4]. Previous studies have reported that the highest ADA activity was observed in the lymphoid and fatty tissues, liver, skeletal muscle, and heart, although the activity was widely distributed in most organs [5,6]. An increase in ADA activity in type 2 diabetic (T2DM) patients has been reported [7-9]. While the mechanism that increases serum and tissue ADA activity is not well known, with higher ADA activity in insulin-sensitive tissues, the level of adenosine, which increases glucose uptake into cells, will be reduced [8]. Thus, if ADA activity is suppressed, insulin sensitivity may be improved, and cellular proliferation, inflammation, and T-cell activity, all of which are associated with the pathophysiology of insulin resistance, can also be affected. Therefore, insulin resistance may have an important relationship with ADA activity. However, it is difficult to conclude whether changes in ADA activity are the cause or result of actual insulin resistance [9, 10]. In addition to its association with diabetes, serum ADA activity is also increased in patients with liver cirrhosis as well as in patient with PCOS and infectious diseases such as hepatitis, tuberculosis, brucellosis, and typhoid fever [11, 12].

Since ADA activity is associated with insulin resistance, in the present study, we measured serum ADA activity in PCOS patients with or without metformin therapy to check if metformin as, insulin sensitizer, affect ADA activity in PCOS patients.

Materials & Methods:

Subjects: the study was a cross-sectional study carried out at Obstetric Department at Al-Yarmouk Teaching Hospital, during the period from October, 2011 till the end of September, 2012. The diagnosis of PCOS was made from the history of chronic oligomenorrhoea (cycle length>35 days, or less than 9 cycles per year), amenorrhoea (cycle length> 12wks), infertility with hirsutism or acne, and with an ultrasonographic findings of polycystic ovaries [13]

Exclusion conditions included the following systemic and endocrine disorders: late-onset congenital adrenal hyperplasia, Cushing's syndrome, thyroid dysfunction, hyperprolactinemia, diabetes mellitus, coronary artery disease, and spontaneous abortion. Furthermore, subjects accepting treatment with medications known to alter insulin hemodynamics, ovulation induction, anti-obesity, or oral contraceptives (OCs) within 3 months were excluded from the study. All subjects were nonsmokers, and none reported chronic alcohol consumption. The protocol for the study was approved by the Ethical committee of Al-Nahrain Medical College, and informed signed consent was given by each subject.

A total of 80 patients with PCOS were enrolled in this study: forty of them were newly diagnosed to have PCOS who receives no therapy for PCOS (G1); the remaining 40 patients were women with PCOS who receive Metformin 500 mg (bid) for 90 days as a therapy (G3) as in Table 1.

The study included another 40 apparently healthy subjects, they were neither alcoholic nor smoker with no family history of any type of DM who serve as healthy controls; they were matched with patients` groups for age as in *table 1*.

Blood samples: five milliliters of random venous blood were withdrawn from each patient, in supine position, without application of tourniquet. Samples were transferred into clean new plane tube, left at room temperature for 15 minutes for clotting, centrifuged at 1800 x g for 10 minutes at 4°C, and the separated serum was transferred into Eppendrof tube and was used for measurement of ADA. The tubes were stored at -20° C until analysis, which was done within one month after collection. [14]

Methods: measurement of serum ADA was done by ELISA kit [14].

Statistical analysis: statistical analysis was done using Excel system version 2003 and includes descriptive statistics (mean and standard deviation) and inferential statistics (*t-test*) to test the significancy of mean

difference. When P-value was less than 0.05, the difference is considered statistically significant, and the difference is considered highly significant when P-value was less than 0.001.

Results & Discussion:

Serum Adenosine deaminase: Serum ADA was highly significantly reduced in PCOS group who receive treatment with metformin (G2) when compared with newly diagnosed PCOS group whom receive no therapy (G1) [P < 0.001]; however, no significant difference was found when compared with healthy controls (G3) [P > 0.05] despite the high significant elevation of ADA activity which was observed when newly diagnosed PCOS patients (G1) compared with healthy controls (G3) [P < 0.001] as in *Table 2*.

PCOS has been a subject of research and debate over past six decades. Insulin resistance accompanied by compensatory hyperinsulinemia is a common feature of PCOS [15].

Because ADA is closely related to T lymphocyte function [16] insulin and resistance, in the present study, we measured ADA activity in PCOS patients to evaluate this enzyme and to demonstrate whether ADA activity is affected by therapeutic drug (metformin). According to our results, ADA activity in PCOS patients was significantly higher than that in the control group, ADA activity comparisons showed that ADA activity was significantly lower in the metformin group than it was in the PCOS group; newly diagnosed however, no significant increase was found when

metformin treated group compared with the control group. Metformin decreases insulin resistance, so ADA activity is expected to decrease in conjunction with metformin therapy. Reports from one study conducted on red blood cell lysates showed that metformin did not directly inhibit ADA activity [17]; however, the exact mechanism still remains unclear. Metformin's glucose-lowering effects and various other effects such as an anti-inflammatory actions. T-cell differentiation inhibition, TNF- α inhibition, and other immune regulatory effects [18] may be considered to have an effect on ADA activity.

In line with previous reports done by other researchers, ADA activity in PCOS patients in the present study are consistent with those reported by Hoshino et al. [19]. The limitation of this study was as follows: 1) ADA activity differences based on treatment and were compared in PCOS patients through a simple cross-sectional study, 2) comparisons before and after medical treatments were not performed, In conclusion, compared to the control group, ADA activity in newly diagnosed PCOS patients was higher. When metformin therapy was used for 90 days, ADA activity was nearly as controls.

Conclusion:

The results of this study suggest that the metformin currently used by PCOS patients do have an effect on ADA activity indirectly through improving insulin sensitivity. Additional studies are needed to evaluate the

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activity of ADA in various insulin sensitive tissues. *Table (1): Clinical criteria of patients` groups with Polycystic Ovarian Syndrome & Control* (presented as range and mean ± SD).

Group	G1	G2	<i>G3</i>
No	40	40	40
Age / year (Mean + SD)	32 <u>+</u> 3.4	34 <u>+</u> 24	35 <u>+</u> 5
Age range (years)	28-41	31-40	33-41
BMI -(kg/m²) (mean <u>+</u> SD)	21 <u>+</u> 4.8	27.2 <u>+</u> 4.5	22.1 <u>+</u> 4.5
BMI Range(kg/m²)	16-23.9	22.4-34.7	15-26

(G1): women with PCOS: newly diagnosed, on no treatment.

(G2): women with PCOS: on metformin therapy 500mg (bid) for 90 days.

(G3): HealthyControls.

Table (2): The mean serum Adenosine deaminase in different women with Polycystic OvarianSyndrome and controls (presented as mean ± SD).

Variable	G1	G2	G3
serum ADA (IU/L)	26.4 <u>+</u> 4.9* [§]	17.9 <u>+</u> 3.5**	14 <u>+</u> 2.8

(G1): women with PCOS: newly diagnosed, on no treatment.

(G2): women with PCOS: on metformin therapy 500mg (bid) for 90 days.

(G3):HealthyControls.

* t-test: G1 versus G2, p < 0.001

** t-test: G2 versus G3, p<0.05

§t-test: G1 versus G3, p<0.001

The authors declare that they have no conflict of interest.

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Production of Digital Climatic Maps Using Geostatistical Techniques

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Abstract:

There is an increasing demand for girded datasets of climate variables from fields such as hydrology, ecology, agriculture, climate change research and climate model verification. The girded climate data sets developed are very suitable for digital data storage and access. The temperature is the most important climatic elements. It effects on the various human activities. There is a mutual relationship between temperature and climate. It is the base motivation engine for the rest of the climate elements. Consequently this paper attempted to make spatial interpolation, of annual and monthly maximum temperature in Iraq for the period from 1970 to 2010 using spatial geostatistics tools in ArcGIS Version 9.3. This paper presents a methodology to produce accurate climatic maps. Validation of produced maps was examined by different criteria.

Keywords: Geographic information system, Geostatistical analyst, Kriging, Temperature.

إنتاج خرائط المناخ الرقمية باستخدام التقانات الجيواحصائية

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الخلاصة:

يوجد طلب متزايد على مجاميع البيانات الشبكية للمتغيرات المناخية من حقول المعرفة المتعددة متل الهيدرولوجي ،علم البيئة ،الزراعة ، بحوث التغيرات المناخية ،والتحقق من موديلات المناخ. إن مجاميع البيانات المناخية الشبكية المطورة تكون مناسبة للغاية لأغراض خزن البيانات الرقمية والوصول إليها ، و تعد درجة الحرارة أكثر عناصر المناخ أهمية بسبب تأثيرها على النشاطات البشرية المختلفة. توجد علاقات متبادلة بين درجة الحرارة والمناخ لكونها المحرك الأساس لبقية عناصر المناخ و نتيجة لذلك فان البحث الحالي يحاول أن يعمل استنباط مكاني لمعدلات درجة الحرارة العظمى الشهرية والسنوية بالعراق للفترة من 1970 ولغاية 2010 باستخدام الأدوات الجيواحصائية المكانية في برنامج العظمى الشهرية والسنوية بالعراق للفترة من 1970 ولغاية 2010 باستخدام الأدوات الجيواحصائية المكانية في برنامج منتافة.

كلمات مفتاحيه : نظام المعلومات الجغرافي،المحلل الجيواحصائي ،kriging ،درجة الحرارة.

INTRODUCTION:

Estimates of the spatial distribution of climatic variablesare than for required more ever sustainable management of natural resources. Determining spatial climateconditions, however, is not because long-term easy, average observations come weather from discreteand irregularly sparse, distributed meteorological stations. These discrete data has been extended spatially to reflect the continuously and gradually changed climate pattern. Climate monitoring requires an operational analysis of the variability of climatic quantities in space and time. For this purpose, operational maps generated for regular time intervals (days, months, seasons, years) It is very useful to see at a glance the spatial variability of climate elements and change with time. Such maps are often used by national meteorological and hydrological services as a basis for climate reviews and interpretation of outstanding features of climate variability. Maps have been available for various spatial areas from the catchment scale to the whole globe. Usually, maps are a result of gridding or spatial interpolation of point data into the area. Nowadays, a large variety of mathematical and geostatistical methods for spatial

interpolation is available. The choice of the gridding method depends on the selected area and the selected climate element as well [1 - 3]. Spatial climate mapping is a basic application of thedata sets. Climate distribution can be characterized and displayed cell by cell using GIS, then converted andsaved in а computer-based photograph format for further view. A large number of papers dealing with spatial interpolation of climate data have already been published and an overview of spatial interpolation methods and their application in climatology by GIS software, and many related papers [3 - 6]. More attention has been given to the application of interpolation techniques to climatic analysis in recentyears.Several interpolation approaches are available in geographical information systems (GISs) the to meet general requirements of interpolation. Several interpolation approaches have been used for spatial climatic analysis [7 - 9]. This paper refers specifically to interpolation of monthly spatial maximum temperature for the period 1970 to 2010. The main goal of this paper is to propose an optimal method of spatial interpolation of monthly maximum temperature data, and to examine the validity of the produced maps by calculating different criteria.

MATERIALS and METHODS

The maximum temperature was computed using data from Iragi meteorological organization and seismology, climate department for different period of different stations extended from 1970 to 2010 divided to 3 stages each one extend to 30 years excepts third one cover 20 years. Missing value for any monthly mean was substituted by the mean of maximum temperature for the same periods. The original data is in whole degree centigrade and is computed to tenth of a degree centigrade. The mean monthly value was computed by taking the 30years mean of the monthly means. The monthly means were computed from the daily values of maximum temperature. The mean annual value was computed by taking 30 years mean of the yearly means. The yearly means were computed by averaging their 12 monthly mean values. Three periods were covered; from 1970 to 2000, from 1980 to 2010, and the third period from 1990 to Due to non-availability 2010. of abundant measurement points, reliable estimation of temperature distribution poses a great challenge.

The spatial interpolation prediction techniques (like spline, inverse distance weighting and kriging)

provide better estimation of temperature than conventional methods [10 - 11]. There is'nt single method for preferred data interpolation which can meet with the selection criteria of required level of accuracy, the time and/or computer resources etc. The common approach to select the optimal spatial interpolation method has become the focus. To determine the validity of interpolated temperature maps by using statistical criteria and subjective comments.Various spatial and statistical tools were used to display and analyze trendsin temperature data. In this paper ArcGIS has been used to produce the spatially distributed temperature data by using Kriging method.

3 - Kriging Theory:

The presence of a spatial structure where observations close to each other are more alike than those that farapart (spatial are autocorrelation) is a prerequisite to the application of geostatistics. The experimental variogram measures the average degree of dissimilarity between unsampled values and a nearby data value, and thus can depict autocorrelation at various distances [9 , 12].

The value of the experimental variogram for a separation distance of h (referred to as the lag) is half the

$$\hat{\gamma}(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [z(x_i) - z(x_i + h)]^2$$

Where N(h) is the number of data pairs within a given class of distance and direction. If the values at z(xi) and z (xi + h) are auto correlated the result small. will be relative to an uncorrelated pair of points. From the analysis of the experimental variogram, a suitable model (e.g. spherical, exponential) is then fitted, usually by weighted least squares, and the parameters (e.g. range, nugget and sill) are then used in the kriging procedure.

average squared difference between the value at z(xi) and the value at z(xi) + h[6]:

(1)

variogram The must be expressed as a mathematical function before being used for kriging. This is typically achieved by fitting a suitable function to the experimental variogram. Each function is defined in terms of a small number of parameters that are selected to best-fit the function to the experimental variogram. In this study we use two functions, namely spherical and circular. Below the spherical function adopted, which is defined by[6]:

$$\gamma(h) = \begin{cases} c_0 & \text{when } h = \varepsilon \text{ (a very small lag)} \\ c_0 + c(\frac{3h}{2a} - \frac{1}{2}(\frac{h}{a})^3) & \text{when } 0 < h \le a \\ c_0 + c & \text{when } h > a \end{cases}$$
(2)

where c0 is the nugget variance, c+c0 is sill, h is the lag and a is the range. All variograms computed in this study are all fitted with spherical model.

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The spherical model is the most commonly used model for experimental data [3, 6]. This function is expressed in terms of three parameters, namely; a range of spatial correlation, c0 the nugget effect and c1 the sill value. When a variogram is plotted using discrete experimental data points, it is called an experimental or sample variogram. A theoretical model can be fitted through the experimental data points to quantify spatial patterns. The shape and description of a "classic" variogram [8, 13, 14] is shown in Figure (1).

Lag between observations/m

There are three key terms in each model, the sill, therange, and nugget variance. The sill corresponds to theoverall variance in the dataset and the range is themaximum distance of spatial autocorrelation. Thenugget variance is the positive intercept of the variogramand can be caused by measurement errors or spatialsources of variation at distances smaller than thesampling interval or both.

5-RESULTS and DISCUSSION:

Figures (2) to (7) shows the maps of maximum temperature obtained from weather stations, produced by applying Kriging interpolation method. The functions used for modeling the variogram are the spherical and circular. The results are shown in the figures below. Two months have been chosen, namely January and July, for the three periods from 1970 2010. to

Figure (2): Maximum Temperature, First Period, January, Using Ordinary Kriging, Spherical Model.

Figure (3): Maximum Temperature, First Period, July, Using Using Ordinary Kriging, Spherical Model.

Figure (4): Maximum Temperature, Second Period, January, Using Ordinary Kriging, Spherical Model.

Figure (5): Maximum Temperature, Second Period, July, Using Ordinary Kriging, Spherical Model.

Figure (6): Maximum Temperature, Third Period, January, Using Ordinary Kriging, Spherical Model.

Figure (7): Maximum Temperature, Third Period, July, Using Ordinary Kriging, Spherical Model.

The main goal of interpolation is to discern the spatial patterns of maximum temperature by estimating values at unsampledlocations based on measurements at sample points.Geostatistics provides an advanced methodology to quantifythe features spatial of the studied variables enables and spatialinterpolation, kriging. In addition, geographicalinformation systems (GIS) and geostatistics have opened upnew ways to study and analyze spatial distributions ofregionalized variables, distributed continuously on space. Moreover, they have become useful tools for thestudy of hazard assessment and spatial uncertainty.Without a GIS, analysis and management of large spatial databases may not be possible. Since a strong spatial dependence between maximum temperature data is observed, the geostatistical algorithms,

particularly theordinary kriging, provide accurate estimates.

CONCLUSION:

Creation of digital grid maps makes it possible to obtain climatic information at any point, whether there is a weather station or not. Multiple factors condition the difficulty of map creation, such as the location of the site samples, spatial density, spatial variability etc. Interpolating values of climate variables from measurement stations to large areas istherefore fundamental and requires minimizing the extent of interpolation errors by using a suitable interpolation method. Given a set of meteorological data, it's possible to use a variety of stochasticand deterministic interpolation methods to estimate meteorological variables at unsampledlocations.

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A Proposed Technique for Solving Linear Fractional Programming

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<u>Abstract</u>

Linear fractional programming (LFP) problems are useful tools in production planning, financial and corporate planning, health care and hospital planning and as such have attracted considerable research interest. The paper presents a new approach for solving a fractional linear programming problem in which the objective function is a linear fractional function, while the constraint functions are in the form of linear inequalities. We illustrate a number of numerical examples to demonstrate a proposed technique. We then compared proposed technique with Cooper's method in the literature for solving (LFP) problems

Keyword: Optimal Solution, Linear Fractional Programming , Charnes& Cooper

Transformation, New Approach

طريقة مقترحة لحل أنموذج البرمجة الخطية بشكله الكسري

الخلاصة

إن الأسلوب الكسري للأنموذج الرياضي يعتمد على الأدوات والأساليب الحديثة المهمة في عملية تخطيط الإنتاج ، التخطيط المالي والاقتصادي والرعاية الصحية في المستشفيات، وعلى هذا النحو فقد جذبت اهتماماً وقدراً كبيراً في عملية البحث والتطوير ينفرد البحث بنهج جديد أو تقنيه جديدة لحل مشكلة البرمجة الخطية الكسرية التي تكون دالة الهدف فيها دالة كسرية ، وأما القيود فهي خطية وعلى شكل متراجحات ، ولتوضيح آلية العمل سنقوم بإعطاء أمثلة رقمية لإثبات جودة ودقة التقنية الجديدة مع الأخذ بنظر الاعتبار مقارنتها مع طريقة كوبر من اجل الحصول على الحل الأمثل لمشكلة البرمجة الخطية الكسرية

الكلمات المفتاحية: الحل الأمثل ، البرمجة الخطية الكسرية ، تحويل كوبر ، التقنية الجديدة

1. INTRODUCTION :

In this section, we establish the relationship between LP and LFP problems, and we consider the following (LFP).

(LFP)

Maximize
$$F(x) = \frac{c^T x + \alpha}{d^T x + \beta}$$
.....(1)

Subject to

$$x \in X = \left\{x \in \mathbb{R}^n : Ax \leq b, x \geq 0\right\}$$
.....(2)

Where

A : is a (m*n) Matrix ; c,d,x are(n*1) vectors

b : is an (m*1) vector and α,β are scalars

It is assumed that the feasible regions nonempty and bounded and the denominator

is satisfy $\frac{\beta(Ax - b)}{\beta(d^T x + \beta)} \le 0$ dx+6>0. if dx+6≤0 then the condition

2. Solving Approach :

<u>Approach one</u>: Charnes& Cooper Transformation to linear program, we assume

including two Transformation
$$t = \frac{1}{d^T x + \beta}$$
, $Y = \frac{1}{d^T x + \beta} x$, $Y = tx$ and $x = \frac{Y}{t}$

(i) :Transformation of Objective Function (Z) then

$$F(x) = \frac{c^T x + a}{d^T x + \beta} = t(c^T x + a)$$

$$\therefore x = \frac{Y}{t} \quad then$$

$$F(Y) = t(c^T \frac{Y}{t} + a) = c^T Y + at \dots(3)$$

(ii) :Transformation of Constraints (S.T.)

 $\therefore Ax \le b \to x = \frac{Y}{t} \quad then$ $A\frac{Y}{t} \le b \quad then$ $AY \le bt$ $AY - bt \le 0....(4)$ and $t = \frac{1}{d^T x + \beta} \to t(d^T x + \beta) = 1$ $\therefore t(d^T \frac{Y}{t} + \beta) = 1 \quad then$ $d^T Y + \beta t = 1 \quad(5)$

From above equations(3),(4)and(5).we obtain the new(LP) model from (LFP)model as follow

 $Max \quad F(Y,t) = c^{T}Y + at$ S.T. $AY - bt \leq 0.....\mu$ $d^{T}Y + \beta t = 1....\lambda$ $Y, t \geq 0$

And the dual program is

Max $F(\mu, \lambda) = \lambda$ S.T. $A\mu + d^T \lambda \ge c^T$ $-bY + \beta \lambda = \alpha$ $\mu \ge 0, \lambda \text{ is U.R.S.}$

<u>Approach two</u>: New technique transformation including two transformation

(i) :Transformation of Objective Function (Z)

Multiplying both the denominator and numerator of equation(1) by β , we get

$$F(x) = \frac{\beta(c^{T} x + a)}{\beta(d^{T} x + \beta)} adding \& substruct (d^{T} ax) for the numerator
F(x) = \frac{\beta c^{T} x + d^{T} ax - d^{T} ax + \beta a}{\beta(d^{T} x + \beta)}$$

$$F(x) = \frac{(c^{T} \beta - d^{T} a)x + (d^{T} x + \beta)a}{\beta(d^{T} x + \beta)}$$

$$F(x) = \left(c^{T} - \frac{a}{\beta}d^{T}\right)\frac{x}{(d^{T} x + \beta)} + \frac{a}{\beta}$$
(6)
Put $Y = \frac{x}{(d^{T} x + \beta)}, V = \left(c^{T} - \frac{a}{\beta}d^{T}\right) and P = \frac{a}{\beta}$

$$F(Y) = VY + P \dots(7)$$

(ii) :Transformation of Constraints (S.T.)

Multiplying equation(2) by β , we get

$$\frac{\beta(Ax - b)}{\beta(d^{T}x + \beta)} \leq 0$$

$$\frac{\beta Ax - b\beta}{\beta(d^{T}x + \beta)} \leq 0$$

$$adding \& substruct (d^{T}bx) for the numerator
$$\frac{\beta Ax + d^{T}bx - d^{T}bx - b\beta}{\beta(d^{T}x + \beta)} \leq 0$$

$$\frac{\beta\left(Ax + d^{T}\frac{b}{\beta}\right)x}{\beta(d^{T}x + \beta)} - \frac{b}{\beta}\left(\frac{d^{T}x + \beta}{d^{T}x + \beta}\right) \leq 0$$

$$\left(Ax + d^{T}\frac{b}{\beta}\right)\frac{x}{d^{T}x + \beta} \leq \frac{b}{\beta}$$

$$Put \ G = \left(Ax + d^{T}\frac{b}{\beta}\right), \ Y = \frac{x}{d^{T}x + \beta}, \ H = \frac{b}{\beta}$$

$$GY \leq H.....(9)$$$$

From above equations(7)and(9).we obtain the new(LP) model from (LFP)model as follow

$$F(Y) = VY + P$$

S.T.
$$GY \le H$$

$$Y \ge 0$$

3. Numerical Examples

In this section, we will illustrate some numerical examples to demonstrate approaches.

Example (1) : (Charnes&Cooper approach)

$$Max \quad Z = \frac{x_1 + 2x_2}{2x_1 - x_2 + 2}$$

S.T. $-x_1 + 2x_2 \le 2$
 $x_1 + x_2 \le 4$
 $x_1, x_2 \ge 0$

Let
$$t = \frac{1}{2x_1 - x_2 + 2}$$
, $Y_1 = tx_1 \rightarrow x_1 = \frac{Y_1}{t}$
 $Y_2 = tx_2 \rightarrow x_2 = \frac{Y_2}{t}$

Then the new(Z) is

$$F(Y,t) = t \left(\frac{Y_1}{t} + 2\frac{Y_2}{t}\right)$$

 $F(Y,t) = Y_1 + 2Y_2$

And the new constraints are

S.T

$$\rightarrow \left(-\frac{Y_1}{t} + 2\frac{Y_2}{t} \right) \le 2$$

$$\rightarrow -Y_1 + 2Y_2 \le 2t$$

 $-Y_1+2Y_2-2t\leq 0$

$$\rightarrow \left(\frac{Y_1}{t} + \frac{Y_2}{t}\right) \leq 4$$
$$\rightarrow \quad Y_1 + Y_2 \leq 4t$$

 $Y_1+Y_2-4t\leq 0$

Also

$$\rightarrow \left(2\frac{Y_1}{t}-\frac{Y_2}{t}+2\right)=1$$

 $2Y_1 - Y_2 + 2t = 1$

So, the new (LP) model is

$$F(Y,t) = Y_1 + 2Y_2$$

S.T. $-Y_1 + 2Y_2 - 2t \le 0$
 $Y_1 + Y_2 - 4t \le 0$
 $2Y_1 - Y_2 + 2t = 1$
 $Y, t \ge 0$

The initial basic feasible solution is given bellow

		1	2	0	0	0	-M
B.V	X _B	Y ₁	Y ₂	t	S ₁	S ₂	R ₁
S ₁	0	-1	2	-2	1		
S ₂	0	1	1	-4		1	
R ₁	1	2	-1	2			1
F(Y,t)	-M	1	2	0			

Tableau(1)

And the optimal solution is given bellow

B.V	X _B	1	2	0	0	0	-M
		Y ₁	Y ₂	t	S ₁	S ₂	R ₁
Y ₂	1	1	1		1		1
S ₂	3	6			1	1	3
т	1	3/2		1	1/2		1
F(Y,t)	2	1			2		M+2

Tableau(6)

Now

$$x_{1} = \frac{Y_{1}}{t} = \frac{0}{1} = 0$$

$$x_{2} = \frac{Y_{2}}{t} = \frac{1}{1} = 1$$

$$Z(x) = \frac{x_{1} + 2x_{2}}{2x_{1} - x_{2} + 2} = \frac{(0) + 2(1)}{2(0) - (1) + 2} = 2$$

Example (2) :(Charnes&Cooper approach)

$$Max \quad Z = \frac{5x_{1} + 6x_{2}}{2x_{2} + 7}$$

S.T. $2x_{1} + 3x_{2} \le 6$
 $2x_{1} + x_{2} \le 3$
 $x_{1}, x_{2} \ge 0$

Let
$$t = \frac{1}{2x_2 + 7}$$
, $Y_1 = tx_1 \rightarrow x_1 = \frac{Y_1}{t}$
 $Y_2 = tx_2 \rightarrow x_2 = \frac{Y_2}{t}$

Then the new(Z) is

$$F(Y,t) = t \left(5 \frac{Y_1}{t} + 6 \frac{Y_2}{t} \right)$$

 $F(Y,t) = 5Y_1 + 6Y_2$

And the new constraints are

$$S.T$$

$$\rightarrow \left(2\frac{Y_1}{t} + 3\frac{Y_2}{t}\right) \leq 6$$

$$\rightarrow 2Y_1 + 3Y_2 \leq 6t$$

 $2Y_1+3Y_2-6t\leq 0$

$$\rightarrow \left(2\frac{Y_1}{t} + \frac{Y_2}{t}\right) \leq 3$$
$$\rightarrow 2Y_1 + Y_2 \leq 3t$$

 $2Y_1+Y_2-3t\leq 0$

Also

$$\rightarrow \left(2\frac{Y_2}{t}+7\right)=1$$

 $2Y_2 + 7t = 1$

So, the new (LP) model is

$$F(Y,t) = 5Y_1 + 6Y_2$$

S.T. $2Y_1 + 3Y_2 - 6t \le 0$
 $2Y_1 + Y_2 - 3t \le 0$
 $2Y_2 + 7t = 1$
 $Y, t \ge 0$

The initial basic feasible solution is given bellow

			Table	au(1)			
B.V	X _B	5	6	0	0	0	-M
		Y ₁	Y ₂	t	S ₁	S ₂	R ₁
S ₁	0	2	3	-6	1		
S ₂	0	2	1	-3		1	
R ₁	1	0	2	7			1
F(Y,t)	-M	-5	-6	0			

And the optimal solution is given bellow

			Table	au(5)			
B.V	X _B	5	6	0	0	0	-M
		Y ₁	Y ₂	t	S ₁	S ₂	R ₁
Y ₂	0.15		1		0.35	-0.35	0.15
Y ₁	0.075	1			-0.325	0.825	0.075
т	0.10			1	-0.1	0.1	0.1
F(Y,t)	1.275				0.475	2.025	1.275+M

Now

$$x_{1} = \frac{Y_{1}}{t} = \frac{0.075}{0.10} = 0.75 = \frac{3}{4}$$

$$x_{2} = \frac{Y_{2}}{t} = \frac{0.15}{0.10} = 1.5 = \frac{3}{2}$$

$$Z(x) = \frac{5x_{1} + 6x_{2}}{2x_{1} + 7} = \frac{5\left(\frac{3}{4}\right) + 6\left(\frac{3}{2}\right)}{2\left(\frac{3}{2}\right) + 7} = \frac{51}{40}$$

Resolve Example (1) by using (New technique 1)

$$Max \quad Z = \frac{x_{1} + 2x_{2}}{2x_{1} - x_{2} + 2}$$

S.T. $-x_{1} + 2x_{2} \le 2$
 $x_{1} + x_{2} \le 4$
 $x_{1}, x_{2} \ge 0$

We have

$$c^{T} = (1,2), d^{T} = (2,-1), a = 0, \beta = 2$$

 $A_{I} = (-1,2), b_{I} = 2$
 $A_{I} = (1,1), b_{I} = 4$

Where

A₁& b₁ is related to the first constraint

A₂& b₂ is related to the second constraint

So ,we have the objective function from equation (7)

$$F(Y) = \left[(1,2) - \frac{o}{2}(2,-1) \right] \begin{bmatrix} Y_1 \\ Y_2 \end{bmatrix} + \frac{o}{2}$$

$$F(Y) = Y_1 + 2Y$$

Now the first constraint ,we get from equation(9)

$$\left[(-1,2) + \frac{2}{2}(2,-1) \right] \begin{bmatrix} Y_1 \\ Y_2 \end{bmatrix} \leq \frac{2}{2}$$

 $Y_1 + Y_2 \leq I$

Similarly second constraint ,we get

$$\left[(1,1)+\frac{4}{2}(2,-1)\right]\begin{bmatrix}Y_1\\Y_2\end{bmatrix}\leq\frac{4}{2}$$

$5Y_1-Y_2\leq 2$

So, the new (LP) model is

$$F(Y) = Y_1 + 2Y_2$$

S.T. $Y_1 + Y_2 \le 1$
 $5Y_1 - Y_2 \le 2$
 $Y_1, Y_2 \ge 0$

The tableau(2) is represent the optimal solution (final tableau)is given below

B.V	X _B	1	2	0	0
	b	Y ₁	Y ₂	S ₁	S ₂
Y ₂	1	1	1	1	
S2	4	7		2	1
F(Y)	2	1		2	

Tableau(2)

<u>Resolve Example (2) by using (New technique 1)</u>

Max	$Z = \frac{5x_1 + 6x_2}{2x_2 + 7}$
<i>S.T</i> .	$2x_1+3x_2\leq 6$
	$2x_1 + x_2 \leq 3$
	$x_1, x_2 \ge 0$

We have

$$c^{T} = (5,6), d^{T} = (0,2), \alpha = 0, \beta = 7$$

 $A_{I} = (2,3), b_{I} = 6$
 $A_{I} = (2,1), b_{I} = 3$

Where

A₁& b₁ is related to the first constraint

A₂& b₂ is related to the second constraint

So ,we have the objective function from equation (7)

$$F(Y) = \left[(5,6) - \frac{\theta}{1}(\theta,2) \right] \begin{bmatrix} Y_1 \\ Y_2 \end{bmatrix} + \frac{\theta}{1}$$

 $F(Y) = 5Y_1 + 6Y$

Now the first constraint ,we get from equation(9)

$$\left[(2,3)+\frac{6}{7}(0,2)\right] \begin{bmatrix} Y_1\\Y_2\end{bmatrix} \leq \frac{6}{7}$$

 $14Y_1 + 33Y_2 \leq 6$

Similarly second constraint ,we get

$$\left[(2,1)+\frac{3}{7}(0,2)\right] \begin{bmatrix} Y_1\\Y_2\end{bmatrix} \leq \frac{3}{7}$$

 $14Y_1 + 13Y_2 \leq 3$

<u>So, the new (LP) model is</u>

$$F(Y) = 5Y_1 + 6Y_2$$

S.T. $14Y_1 + 33Y_2 \le 6$
 $14Y_1 + 13Y_2 \le 3$
 $Y_1, Y_2 \ge 0$

The tableau(2) is represent the optimal solution (final tableau)is given below

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B.V	Х _в	5	6	0	0
		Y ₁	Y ₂	S ₁	S ₂
Y ₂	0.15		1	0.05	-0.05
Y ₁	0.075	1		-0.046	0.11
F(Y)	1.275			0.067	0.283

Now

4. COMPARISON

In this section, we give a comparison chart to show the efficiency of new technique and (QM-Software)with the Cooper's procedure. To find the duration of implementation code we use "Run Time" command. We use the following

computer configuration. Processor: x86 Family 6 Model 15 Stepping 13 GenuineIntel 2.00GHZ, Memory(RAM):2.00 GB, System type: X86-based PC

Numerical Examples	Methods	Iteration use	Computer Time taken
1	Cooper's procedure	Six	0.59 sec.
	new technique	two	0.11 sec.
2	Cooper's procedure	Five	0.50 sec.
	new technique	two	0.11 sec.

5.Conclusion

Our aim was to develop an easy technique for solving LFP problems. In this study, we have introduced new technique, which converts the LFP problem into a single LP problem. A method for solving linear fractional functions with constraint functions in the form of linear inequalities is given. The proposed method differs from the earlier methods as it is based upon solving the problem algebraically using the concept new transformation. The method appears simple to solve any linear fractional programming problem of any size. We also compared these results obtained by proposed method with cooper's method.

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Measuring the Rates of Radioactive Contamination and Radiation Doses for Rockwool plant

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Abstract

This research aims to assess the radioactivity for the rock wool plant in the province of Baghdad – Al Taji city through conducting radiological survey for the plant which of six part (crushers , furnaces , machines spinning , materials store and waste site) in addition assessment of radiation doses to the worker who are near smelting furnaces by using portable detectors to know the level of exposure and the contamination resulting from smelting basalt stone operations which the essential material for making the rock wool, its contain the natural radioactivity series such as U-238 and Th-232. The results of radiological survey were conducted showed the presence of a significant increase in the rates of radioactive contamination and the dose rates in the area of smelting furnaces ranged between (11.2-7.81) cps using the FH-40 compared with the rate of natural background 0.75 cps and the rates of radiation doses ranged between (0.65- 0.47) µSv/h using the device RadEye compared with natural background 0.04 μ Sv/h ,while the reading in another site within normal levels . it also collected soil samples from the smelting furnaces and from basalt stone accumulation site accordance to IAEA standard which were measured by using gamma spectrometry (which consist of high -purity germanium detector with efficiency of 30% and resolution 2 keV for energy 1.33 MeV of ⁶⁰Co). The results of laboratory tests for the soil elected absence of a significant increase in the values of the concentration for the showed radionuclide which back to the series of U-238 and Th-23 and showed a natural concentrations for isotope of Radium -226 especially in the samples that have been taken from the smelting furnaces (B1,B2) ranged between (62.23 - 24.1) Bq / kg, respectively, that Ra-226 isotope (1600 year half life) emitting Alpha and Gamma rays and decaying to the Radon gas. Where high concentrations of Radon gas is one of the main reasons causing lung cancer, so it is necessary to use protective equipment needed by workers in these factories.

Keywords: Basalt stone, Rockwool, Radiological survey, Radiation dose rates

قياس معدلات التلوث الإشعاعي والإشعاع جرعات لمصنع الصوف الصخري

أحمد عبد الحسن حسين

الخلاصة :

يهدف هذا البحث الى تقييم النشاط الاشعاعي لمعمل الصوف الصخري الواقع في محافظة بغداد / التاجي من خلال اجراء مسح اشعاعي للمعمل والمتكون من ستةاجزاء (الكسارات ، الافران ، ماكنات الغزل ، المخازن، موقع النفايات وموقع تجميع حجر البازلت) بالاضافة الى تقييم الجرعات الاشعاعية للعاملين باستخدام اجهزة الكشف الاشعاعي المحمولة لغرض معرفة الزيادة الحاصلة في مستويات التعرض والتلوث الاشعاعي الناتجة من عمليات صهر حجر البازلت الذي يعتبر المادة الاساس في صناعة الصوف الصخرى وذلك لاحتوائه على السلاسل الاشعاعية الطبيعية مثل سلسلة اليوراينويم-238 والثوريوم-232. وقد اضهرت نتائج المسوحات الاشعاعية التي اجريت وجود زيادة ملحوضة في معدلات التلوث الاشعاعي في منطقة افران الصبهر تراوحت بين 11.2(PH-40) باستخدام جهاز 40-FHمقارنةً مع معدل الخلفيية الاشعاعية الطبيعية0.75 cps اما معدلات الجرع الاشعاعية تراوحت بين psv/h (0.65-0.47) باستخدام جهاز RadEye مقارنةً مع الخلفية الطبيعية 0.04 µSv/h اما بقية المواقع فكانت القراءات ضمن مستوياتها الطبيعية . كما واخذت نماذج تربة من افران الصبهر ومن موقع تجميع حجر البازلت وفق المعايير والمواصفات المعتمدة عالميا لهذا النوع من قياسات النشاط الاشعاعي. وتم قياساها باستخدام منظومة الجرمانيوم عالى النقاوة ذو كفاءة 30% وقدرة فصل2 keV للطاقة 1.33 MeV لنظير الكوبلت 60°Co ، اظهرت نتائج الفحوصات المختبرية لنماذج التربة الماخوذة عدم وجود زيادة ملحوظة في قيم النشاط الاشعاعي النوعي للنويدات المشعة التي تعود الي سلسلتي اليورانيوم-238 والثوريوم-232 واظهرت تراكيز طبيعية لنظير الراديوم -226 خاصةً في النماذج التي تم اخذها من افران الصهر حيث تراوحت بين Bq/kg (62.23–24.1) حيث يعتبر نظير الراديوم-226 ذو عمر نصف 1600 سنة) من النظائر الباعثة لاشعة الفا وكاما ويتحلل الى غاز الرادون, وحيث ان ارتفاع تراكيز غاز الرادون هو احد الاسباب الرئيسية المسببة لسرطان الرئة لذا فانه من الضروري استخدام المعدات الوقائية اللآزمة من قبل العاملين في هذه المصانع .

الكلمات المفتاحية : حجر البازلت ، الصوف الصخرى ، معدلات الجرعة الاشعاعية

Introduction

Exposed human Since ancient time tonaturalradiationoriginating fromcosmic raysandotherradioactive material werefound inthe Earth's crustsince its creation Asconsisting

ofradionuclideactiveradiationgenerated bythe dissolution alpha particles, beta and gamma andcan enterthese particlesintothe human bodythrough foodorbreathing and the main importantsources of natural exposurearepotassium-40,carbon-14and three important natural chainsareUranium-238, Thorium-232andActinium-235 [1].

Representedradioactivityin sometypes ofrocksradionuclideoriginGround (primordial radionuclide) Such as potassium-40as well as theradioactive elementsof thechainsof Uranium-238and Thorium-232 However therate ofradioactivityresultingfromthe concentration of these elements vary from anotheraccording onetypeto to thesenaturalrocks[2] examples ofthese rocks which typesarebasalt is igneous rocksvolcanicblackcolorcontaining52% ofsilica(SiO2)interferenceina multitude of uses. including pavingroadsrailways, in theornamental stones. shieldsconcrete, pipecorrosion resistance and in the manufacture ofrock wool Material [3].

Rock wool Materialisa natural substancein the form ofinorganicfibersassembled due tothe exposure ofmoltenbasalt rocksof thefastmovingcylinders characterizedby isolatingheat, sound, and highresistancetofire it is also usedin thelining ofthe vessels air conditioning systems from the insideorfrom the outside. In Iraqthere isone factoryto produce thisMaterialwhich is**THAT AL-SAWARI**plant located in theTaji area/province of Baghdad. [4]

2- Description of the plant:

The plant is located in the north-western province of Baghdad/Taji area It is one of the subsidiaries the Ministry of Industry and Minerals It consists offourmain parts, a crushers, smelting furnaces, spinningmachinesand stores. Themanufacturing processes ofmaterialpassfourstages the first phaseiscrushingbasaltstone into small piecesbycrushers steel giant, The second phase is passed small pieces of basaltstonesin to special furnaces which are in fact only two ,the temperature rangefor each furnace furnace (1250)°C between 1110°C)degreesCelsius The third stagethenturnstomoltenbasalt stonewithvery high heatandpour into Fast-movingcylindersin order tobecome in the formofcysticconfluentRockwool Material The fourth stage is passed to the spinning machines and rolling with aluminum platesin order to beready for use.

3-Materials andmethods

Setthe backgroundradiation:

Foridentifying the backgroundradiation of the plantwere measuredrates ofexposure andradioactive contaminationofareasnear the plantusing aportableradiationdetectors 50readinghave been recordedof the siterepresentsthe rate ofexposure andcontaminationat the site . soilsamples were elected from the site of assembly basalt stoneandsmelting furnacesto conductlaboratory analyzesand knowledge of the concentration of the radionuclide.

Radiologicalsurveys:

Conducted radiological surveysfor the plantusingportable devices and depending on the instructions issued by the International Atomic Energy Agency (IAEA) [5] were divided measurement area into squaresaccording to natural and size of the areawhere they were division the plant into six regions crushers area (A), smelting furnaces(B), machiness pinning(C), storeraw materials, (D), wasterock

wool(E),siteassemblybasalt(F)and the fact thatsitefurnacesare the more important of therest

sitesthereforeconductedmeasurementsof radiationfrom the front of slot first furnace, and at a distance 1 meterand 2meters (B1, B1-1, B1-2), respectively, and in the samewayfor the second furnace(B2, B2-1, B2-2).the rates ofradiation exposure weremeasuredat an altitude of1 meterfrom thesurface of the earththroughwalkingslowlyon footwhile the measurements ofradioactive contaminationratesat an altitude of5 cm from thesurface of the earth.

4– Thedevices used:

Rad Eye PRD device : A portable deviceto measure thedoseof radiation which is a sodium iodidedetectorwithhigh sensitivityfor the detection ofthe source ofionizing radiationwith the low level through dose rateradiation thatresulting from exposuretogamma rays provideropticalamplifierallowsfor the detection oflow-levels f radiation. Contains anLCD displaythat showsthe results, and weight 160 grams, size96mm x61mm x31mm.the possibility ofdetectingenergiesrangingfrom(60keV 1.3MeV), consumes little power(battery

number2voltage1.5 V),theunit of measurement μ Sv/h[6].

FH-40 G-L10 device aportable :Is devicewith adigital scalewiththe detector byconnectingan externalcableto detectcontaminationAlpha, BetaandGamma rays unitscps / h.Thisdeviceis one of thetypes ofcountersproportionalityprovidertubeproporti onal counterinternal, has aLCD screenthat showsthe results, weight 410 grams, size195mm x 73 mm x 42mmx,can be separated the detector contamination from the devices that it becomes a device for measuring the rate of radiation doses the extent ofenergiesranging from(33 keV-3MeV)andunits ofµSv /h,consumeslittle power(battery number2voltage1.5 V) [7].

Gamma spectroscopy system has used to measure the concentration of radionuclide in the soil models , which consists of the counter germanium high-purity with the efficiency of30% and the amount of resolution 2keV ,Energy1.33MeV for cobalt-60 isotopeand thedetectoris surrounded protective bya barrierhighefficiencymade by Canberra company U.S. the program uses analytical gamma vision6.8developed, were calibrated energy and efficiency measurement system using a standard source multi-energies (MGS5.1045)withradioactivity1.1 Ciu the radioactivity of soil samplesis measured of after transfer the contentstoa special Beaker)3600 containercalled (Marnelli seconds was chosen astimeto measure themodels[8].

Measurement area

figure(1)the results of measurements of radiation doserates for elected positions at the plant by RadEye device

Radiologicalsurveyswere conducted to measure therates of radioactive contamination of sites elected in the plant by FH-40 device a unit of measurement cps and the results are shown infigure (2)

Figure (2)the results of measurements of radioactive contamination rates for elected positions at the plantby FH-40 device

Figures(1,2) show of measurementsusing aportable radiation detection devices, which the significant referring to lack ofa increasein the rates ofradiationdosesandradioactive contamination that can beexposed to a person whoexist inthose areas exception thefurnaceswhererates of radiation dosereached at the site of the first furnace (B1) 0.65 μ Sv/h and radioactive contamination 11.2 cps, at the second furnace (B2) the rates of radiation doses values reached 0.47 uSv/h andradioactive contamination 7.81 cps all of these values are higher than twice thenaturalbackgroundradiation, which is 0.04 µSv/hfor therates of radiation doses and 0.75 cps for therates of radioactive contamination . Thus the workers who are applythe instructions of the International Atomic Energy inthat locationshould Agencywith regard tothe principles ofradiation protectionto makeexposures within prescribed limits laid downglobally[9].

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To give thereliability and characterization of comprehensive for the radiological surveys and the radioactivity of sites elected were taking samples of soil from the smelting furnaces which are only two furnaces (B1, B2) and site of assembly basalt (F1, F2, F3) these samples measured by using the system analysis of the spectra of gamma rays and the results of the analysis laboratory models elected shapes are shown in (3,4,5,6)

Measurement area

Figure (3)Radioactivity levels for theRadioisotopeinsoilsampleselected

Measurement area

Figure (4) radioactivity levels for isotopepotassium- 40in thesoil samples elected

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There sults of laboratory tests that shown infigures (3,4,5,6) absence of a significant increasein the values of concentration of the radionuclidere sulting from the chains of Uranium-238and Thorium-232. This could be due the presence of a very small amountofcesium-137so as not toexceed2.2 Bq / kg toglobalcascading as a result ofnuclear weapons testsin addition toChernobylaccident and showed a natural concentrations for isotope of Radium -226 especially in models that have been taken from the smelting furnaces (B1,B2) ranged between (62.23 - 24.1) Bq / kg respectively but it is a clear indication of the high readings mobile devices (RadEye and FH40) from natural background in terms ofrates of radiation dosesandradioactive contaminationin thatRadium-226isotopewitha half-lifeof1600 fact yearsforisotopesemittingGamma and Alpha rays which lead dissolutionemission ofRadon gas. Radonis aradioactive gasis colorless, tasteless andodorless, which isanaturalsourceof atomic radiationandis generated in the decomposition of Uranium-238chain. It is only isotope who has the status of gaseous exists in differently concentrationsandin differentplaces. The scientists recently found that the long exposure to high concentrations of radon can lead to lung cancer [10]Especially in places where there is no proper ventilation. This is from one side and the other side the constant exposure for fiber rock wool by inhalation or ingestion without feeling the worker and that for being small in size it causes several diseases, including asbestosis disease, cancer in the cavity surrounding the lungs ,cancer of the larynx, stomach, intestines and rectum [11].

Thus the workers in these plants should wear personal protective equipment PPE (respirators, gloves, suits work disposable, head cover, cover shoes) and provide them personal dosimeter (TLD) examine it regularly in the Ministry of Environment / Center for radiation protection and also must replace workers who are at the site of smelting furnaces continuously in order to keep the limits of radiation doses that prescribed by the International Atomic Energy Agency (IAEA) (1mSv / year) through the apply ALARA base (As low as Reasonably Achievable) [9].

Determine whether dose rates exceed the dose limit for the public of 1mvS/y

0.65µSv/h*8 hr/day * 5day/week * 4week /month *12 month /year

 $1248 \ \mu Sv/year = 1.248 mSv/year > 1 mSv/year$

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