Preparation and Evaluation of Curcuma Longa Loaded Chitosangelatin Composite Films for Wound Healing Activity

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الخلاصة

تستعمل البوليمرات كمادة أساسية لتصميم الأدوية المستعملة لعلاج الأمراض المختلفة .أثبتت كل من مادة الكيتوسين والجيلاتين أمتلاكها مواصفات مناسبة لعلاج الجروح بشكل منفرد وبما أن كلا المادتين تمتلك القدرة على علاج الجروح فأن الدمج بين هذين البوليمرين بمساعدة احد الأدوية المختارة في الفيلم المركب قد يظهر تحسنا في فعالية علاج الجروح .حضر الفيلم المركب باستخدام تركيز ثابت من الكيتوسين وزيادة تركيز الجيلاتين بينما حضر الفيلم المحمل بالنبات الطبي الكركم بزيادة تركيز الكركم فيه.تم تقييم هذه الأفلام من ناحية الكثافة,تحمل الطي, قابلية امتصاص الماء, الفعالية المضادة للجراثيم,قوة الشد, دراسة انتشار الدواء في المختبر ودراسة علاج الجروح في الحيوانات حيث اظهر الفيلم المحمل بنبات الكركم فرقا في تحمل الطي وقابلية امتصاص الماء والفعالية المضادة للجراثيم بالمقارنة مع الفيلم المركب الخالي من الإضافة بينما لم يظهر هناك فرق معنوي في السمك وقوة الشد في الفيلم المحمل بالدواء بالمقارنة مع الأفلام المركبة الخالية من الإضافات النسبة المئوية لتقلص الجروح كانت أعلى للجروح المعالجة بنبات الكركم المحمل على الفيلم المركب بالمقارنة مع الفيلم المركب الخالي أو الغير محمل . من النتائج السابقة نستنتج بأن فيلم الكيتوسين-جيلاتين المحمل بنبات الكركم اظهر قدرة أعلى على علاج الجروح بالمقارنة مع فيلم الكيتوسين-جيلاتين غير المحمل وفيلم الكيتوسين الغير محمل من دون التأثير في قوة الفيلم.

Abstract

Natural polymers are used as lead compounds for design of drugs in treatment of different ailments. Chitosan and gelatin have proven wound healing properties individually. As both have wound healing property, the combination of these two polymers and incorporation of drugs into the composite films may show improvement in wound healing activity. The composite films were prepared with increase in gelatin concentration while curcuma longa loaded films were prepared with increased concentrations of curcuma longa in optimized composite film. These films were evaluated for thickness, folding endurance, water absorption capacity, antibacterial activity, tensile strength, in vitro drug release by diffusion studies and in vivo wound healing studies. The curcuma longa loaded films shown significant difference in folding endurance, water absorption capacity, antibacterial activity when compared to blank composite film. There was no significant difference in thickness and tensile strength of drug loaded films when compared to blank composite films. Percentage of wound contraction was more for wounds treated with curcuma longa loaded composite film than blank composite film. With the above results, it was concluded that curcuma longa loaded chitosan-gelatin composite films had shown more wound healing property than chitosan-gelatin blank composite film and blank chitosan film without interfering in strength of film.

Key Words: *Chitosan , gelatin* **,** *wound healing* **,** *curcuma longa*

Introduction

The primary objective in wound care is the promotion of rapid wound healing with the best functional and cosmetic results. Wound healing may be considered as a specific biological process related to the general phenomenon of growth and regeneration [1]. It is a dynamic process in which normally involves systematic, coordinated and balanced activity of inflammatory, vascular, connective tissue and epithelial cells. The process of wound healing is orchestrated by the extra cellular matrix, while interleukins and other growth factors play a major role in the regulation of cellular processes [2]. Wound dressings are usually used to encourage the various stages of wound healing and create better healing conditions. They

often cover the wound surface to accelerate its healing. be only passive products that had a minimal role in the healing process. The landmark study of Winter [3] in the 1962 initiated the concept of an optimal environment for wound repair and the active involvement of a wound dressing in establishing and maintaining such an optimal environment. This awareness revolutionized the approaches to wound care and paved the way for development of wound dressings from the passive materials to the more functionally active ones [2]. In an ideal condition, a desirable wound dressing should (a) create and keep a moist environment, (b) protect the wound from secondary infections, (c) absorb the wound fluids and exudates, (d) reduce the wound surface necrosis, (e) prevent the wound desiccation, (f) stimulate the growth factors, and also be (g) elastic, (h) non-antigenic and (i) biocompatible [1,2,4]. Based on the types of wounds and modes of healings, numerous materials are developed for use as wound dressing [2,4,5].

Chitosan is a linear polysaccharide composed of randomly distributed β-(1,4)-linked D-Glucosamine and N-acetyl-D-Glucosamine. It is commercially produced by acetylation of chitin, which is structural element in exoskeleton of crustaceans (crabs, shrimps etc). The amino group in the chitosan has a pKa value of 6.5. Thus chitosan is positively charged and soluble acidic to neutral solution, which makes chitosan a bioadhesive and readily binds to negatively charged surfaces such as mucosal membranes. Chitosan is nontoxic, biocompatible [6-8], biodegradable polymer [9]. It has reported uses in drug delivery, cell delivery systems, orthopedics, wound healing [10], ophthalmology, bone healing . It enhances function of polymorpho nuclear cells, macrophages and fibro plastic proliferation of migration . It exhibits antimicrobial activity against bacteria , fungi and yeast. It is hypoallergenic, has rapid blood clotting property, haemostatic and acts as fat attractor by binding dietary lipids [11]. Gelatin is also a natural polymer derived from collagen of animal skin and bones. It is translucent, colorless, brittle and tasteless. It is biodegradable in nature. It has good film forming property and known for its wound healing properties [12] by preventing fluid loss due to exudation . It is commonly used as gelling agent in food, pharmaceutical, photography and cosmetic manufacturing. It is used in manufacture of shells of pharmaceutical capsules. It is used as stabilizer, thickener, texturizer in foods such as ice creams, jams, yogurt, cream cheese. It is used as carrier, coating or separating agent for other substances. Gelatin is used as biological substrate to culture adherent cells. It is a good source of protein [12].

Materials and methods

Materials

Curcuma longa was obtained from local market and purified and then extracted be ethanol 96% according to the method of [13].

Methods

Preparation of film: According to [12**].**

Preparation of chitosan film: The chitosan films was prepared by casting chitosan (which is collected from crab shell [14] (1%) solution in the plastic tray and air dried at room temperature.

Preparation of gelatin film: The gelatin films was prepared by casting gelatin (10%) solution in the plastic tray and air dried at room temperature.

Preparation of chitosan-gelatin composite films: All the chitosan-gelatin composite films (of 6 inch length and 4.5 inch width) were prepared by solvent casting technique. Chitosan (1%) and gelatin (10%) solutions were prepared individually. They were mixed in the ratios of 1:10, 1:20, 1:30, and 1:40. The polymer solution was mixed thoroughly and 0.2ml. of ethylene glycol was added as plasticizer. The solution was filtered under vacuum for the removal of any entrapped air bubbles. Then the solution was cast into the film on the plastic tray and air dried at room temperature (15).(Table-1)
Based on the tensile strength of the films, the ratio of 1:40 was selected for further process.
The drug loaded films were prepared by using this ratio.

Preparation of *Curcuma longa* loaded chitosan-gelatin composite films: Stock solutions of ethanolic extract of *Curcuma longa* were prepared in the concentrations of (400)mg/ml (prepared by add 10 ml from Ethanol 96% to 4 gm of the plant extract) and the second concentration is 200 mg/ml(prepared by taking 4ml from the first dilution and we add 4 ml of ethanol 96% to it ,the third dilution 100 mg/ml (prepared by taking 2ml from the second dilution and add 2 ml of ethanol) the fourth dilution is 50%(made by taking 2 ml from the fourth dilution and add 2 ml of ethanol 96 % to it). From each concentration 0.86ml. of medicinal plant ethanolic extract solution was added to the polymer solution of 1:40 ratio of chitosan and gelatin such that 10, 20, 30, 40 µg. of plant was present in 0.19 sq.cm. area of the film respectively (this area is equal to the area of standard antibiotic disc). Different drug loaded composite films were prepared with composition as shown in **Table 1**.

Evaluation of films

Thickness: The thickness of film influence the time required to absorb the polymer into the body. To determine the uniformity in thickness of film and change in thickness film after drug loading, it was measured for each film using screw gauge at three different sites of the film and the mean was calculated.

Folding endurance: The folding endurance was determined to determine flexibility of film. The flexibility of the film is needed to handle the film easily and for comfortable, secured application of film on the wound. It was determined by repeatedly folding one film at same place till it breaks or folded up to 300 times manually. The number of times of film could be folded at the same place without breaking give the value of folding endurance.

Water absorption capacity: It is of utmost importance, if they are used for biological applications and wound healing. It is used to measure the capacity of blank and drug loaded films to absorb wound exudates. Preweighed, one inch film was placed in 15ml. of distilled water and the weight of the film was noted periodically at first hour, second hour, third hour and 24th hour. Every time after noting the weight, the film was placed in fresh water. Water absorption capacity of the film was determined in triplicate and calculated using a formula Initial weight

% Water absorption capacity =(Final weight - Initial weight/ Initial weight)*100

Antibacterial activity: The prepared films were evaluated for antibacterial activity against four different strains with agar plate diffusion method by measuring the zone of growth inhibition of micro organisms. The antimicrobial activity of a compound is expressed in terms for ability to inhibit growth of bacteria in nutrient agar.

Tensile strength: Tensile strength measures the ability of film to withstand rupture, mechanical pressures or the force required for break the film. Tensile strength of the film was determined by using the *Instron* tensile testing machine at SDDC section in CLRI. It was expressed in MPa units. Tensile strength was done for all composite films.

Diffusion studies: Diffusion studies were conducted with selected medicinal plant loaded films CF14 to find the time taken to release total plant. The diffusion studies were carried out using diffusion membrane. For every 10 minutes, 5ml. of sample was withdrawn from the beaker. The concentration of medicinal plant in the samples was estimated using UV/Visible spectrophotometer at respective wavelength. An equal volume of fresh distilled water was replaced after with drawl of each sample.

In vivo studies: Pathogen free adult female albino rats weighing 150-200 gm. were selected. The rats were housed in polypropylene cages under standard laboratory conditions with 12-hour light dark cycle. The rats were fed with standard laboratory chow (Hindustan Lever limited, Mumbai) and water ad libitum. The wound healing activity was conducted with the protocol as shown in Table .2. The anaesthetized animal was placed on the operation table in normal position. The dorsal fur of the animals was shaved with an electric clipper and the anticipated area (2 cms. length and 1cm.width) of the wound to be created was outlined on the back of the animals on interscapular region i.e, 5mm. away from ears. Full thickness skin from the demarked area was excised to get a wound area of 2sq.cm. After achieving haemostasis, the wound was blotted with sterile gauze in control group, the respective film on the wound of animals in treatment groups (Group-II, III). Then the following parameters were determined at specific time intervals.

Percentage of wound contraction: Wound healing is a process by which damaged tissue is restored as closely as possible to its normal state and wound contraction is the process of shrinkage of area of the wound. It mainly depends on the repairing ability of tissue which may be reduced due to infections. It was measured to find the extent of reduction in wound area at different periods of treatment by graphical method. Wound area was calculated on 7th, 14th, 21st and 28th post wounding day by counting number of squares of retraced wound area on graph paper. The degree of wound healing was calculated as % closure of the wound area from the original wound using a formula:

$$\% \text{ Closure} = 1-(Ad/A0) \times 100$$

(A0 – Wound area on day zero, Ad – Wound area on corresponding days).

Statistical Analysis: The results are expressed as mean ± S.D. Statistical analysis was performed by paired t-test, one-way and two-way analysis of variance (ANOVA) test for multiple comparisons. Statistical significance was set accordingly at p (0.05) level.

Results and Discussion

The thickness of blank and Curcuma loaded films at different compositions of gelatin and Curcuma is shown in **Table 4**. All the films have uniform thickness throughout film. The thickness of all films was between 35-130μm. It was found that increase in the concentration of gelatin, the thickness of composite films was significantly ($p<0.05$) increased. There was no significant difference in thickness of Curcuma loaded films when compared to its respective blank composite film indicated that the Curcuma loading has no influence on the thickness of film**.**

Table 4 shows the folding endurance values of all prepared films. With increase in concentration of gelatin, there was significant increase in folding endurance of composite films. There was significant difference ($p<0.05$) in folding endurance between optimized blank composite film F6 and the Curcuma loaded films may be due to alteration of flexibility of films by drugs. GF14 shown maximum folding endurance among Curcuma loaded films, which indicated that it may have maximum flexibility as shown in **Table 4**.

The water absorption capacity of composite films was significantly increased with increase in gelatin concentration, which may be due to hydrophilic and swelling properties of gelatin [16] as shown in **Table 4**. A composite film F6 has shown maximum water absorption capacity among all the prepared composite films. The water absorption capacity of Curcuma loaded films was not significantly different with change in concentration of drug. It was found that, there was significant difference in water absorption capacity of blank composite films and Curcuma loaded films**.** It indicated that the drug is decreasing the water absorption capacity

of film which may be due to interference of drug in water absorption by polymers. Among Curcuma loaded films, CF10 shown maximum water absorption capacity.

The zone of inhibition with various films against four different bacterial species was different in agar disc diffusion technique as shown in **Table 5**. There was no inhibition of growth with gelatin film (F2), which may indicate that the gelatin is a protein and cannot inhibit the growth or may promote the growth of bacteria. Zone of inhibition with composite film (F6) was less than with the chitosan film (F1) may be due to gelatin incorporation. As the concentration of drug in the film was increasing the mean diameter of zone of inhibition was also increased with drug loaded films. Among Curcuma loaded films CF10 shown maximum inhibition against all tested bacteria. The antibacterial activity of Curcuma loaded films was more than the blank chitosan and composite films. It confirmed that increased antibacterial activity of the Curcuma loaded films than blank films, so drug loaded films can show fast wound healing property than blank films.

Table 6 shows the tensile strength parameters of composite films and selected Curcuma loaded film. As gelatin proportion is increased, parameters like maximum extension, elongation at break (%) and tensile strength were increased. As per paired t-test of statistical analysis there was no significant difference ($p<0.05$) in tensile strength of blank and Curcuma loaded films. It indicated that the tensile strength of film was not changed significantly after loading the drug into film. The percentage of drug release from the selected drug loaded film (CF10) was determined by *in vitro* diffusion studies to find the time taken by film to release the complete loaded drug for eliciting its antibacterial action on wound. 98% of Curcuma was released within 1 ½ hour (90 minutes) indicated that the film is not interfering in drug release on wound as shown in **Table 6.**

In vivo* studies*: Table 8 shows percentage wound contraction of different groups at different time intervals. There was significant ($p<0.05$) difference in percentage of wound contraction between untreated group and treated group (Group-III, IV, V). 100% of wound contraction was observed in groups treated with selected drug loaded film within 28 days. There was significant difference in percentage of wound contraction between wounds treated with chitosan (Group-III), wounds treated with optimized blank composite (Group-IV) and drug loaded films (Group-V). It indicated that chitosan-gelatin composite films and drug loaded chitosan-gelatin films have improved wound healing activity than chitosan alone film. Though there was increased wound contraction with drug loaded films, but no significant difference was observed between drug loaded composite films and blank composite film. Restoration and recovery of cells was observed in wounds treated with chitosan film, chitosan gelatin composite film when compared to untreated wounds in 28th day photomicrographs.

Decrease in wound size was observed in wounds treated with Curcuma loaded films on 28th day when compared to all other groups. This suggested that Curcuma loaded films may have more capacity for fast recovery and rapid epithelialization of skin than in the untreated and

wounds treated with other films (**Figure 1**). It may be due to its antibacterial action which prevents further infections on wound supporting for fast epithelialization by stimulation of wound healing environment. On 7th, 14th and 28th day more decrease in wound size was observed in group treated with chitosan-gelatin composite film when compared to in groups treated with chitosan alone. It indicated that the combination of chitosan-gelatin showed better wound healing property than chitosan alone. On 7th, 14th and 28th day the size of wound was more decreased in group treated with Curcuma loaded film when compared to other groups on respective days which indicated that loading of drug into composite films augmented the healing of wound than blank composite films (Figure .3). This may be due to broad antibacterial activity of Curcuma which reduces infections and thus fastens the healing of wound.

Table 1: Composition of Blank Films

S. No	Code of film	No. of parts	
		Chitosan Films	Gelatin
1	F1	1	-
2	F2	-	1
	Composite films		
3	F3	1	10
4	F4	1	20
5	F5	1	30
6	F6	1	40

Table 2: Composition of *Curcuma longa* Loaded Films

S.NO	Code of Film	µg of plant /0.19 sq.cm
	Curcuma longa	
1	CF7	10
2	CF8	20
3	CF9	30
4	CF10	40

Table 3: Protocol for *In Vivo* Wound Healing Studies

S. NO	Group No.	Purpose	Code Of Film Used for Application
1	I	Normal control	-
2	II	Wound control	-
3	III	Treatment group with blank chitosan film	F1
4	IV	Treatment group with blank Composite film	F6
5	V	Treatment group with Curcuma longa loaded composite film	CF10

Table 4: Thickness, Folding Endurance, Water Absorption Capacity of Prepared Films

Water absorption capacity (%) (Mean±S.D)	Folding endurance (Mean±S.D)	Thickness (µm.) (Mean± S.D.)	Code of film	S.No.
	Blank films			
1101.87±9.08	>300	60±0.017	F1	1
It was dissolved.	177.6±6.55	126±0.02	F2	2
879.60±14.92	202±5.50	30±0.01	F3	3
951.18±7.92	215±3.60	43±0.005	F4	4
1266.69±8.86	225±3.51	56±0.011	F5	5
1411.03±11.99	256±5.50	61±0.01	F6	6
	Curcuma longa loaded films			
1077.58±12.32	206.5±3.4	60±0.01	F7	7
1066.12±18.92	222±4.5	63±0.15	F8	8
1109.68±15.43	225.8±6.5	74±0.02	F9	9
1164.05±18.26	249.15±4.6	69±0.011	F10	10

Table 5: Antibacterial Activity Of Optimized Composite And Curcuma Longa Loaded Films Against Different Organisms

Diameter Of Zone Of Inhibition (In Cms) (Mean±S.D.)				Code Of Film
Pseudomonas Aeruginosa	**Escherichia Coli**	**Staphylococcus Aureus**	**Bacillus Subtilis**	
		Blank Films		
1.52±0.040	1.5±0.072	1.66±0.09	1.70±0.045	F1
0	0	0	0	F2
1.26±0.040	1.20±0.050	1.37±0.060	1.38±0.061	F6
		Curcuma Longa Loaded Films		
2.09±0.050	2.20±0.065	2.33± 0.035	2.26±0.045	CF7
2.45±0.065	2.39±0.050	2.60±0.055	2.52±0.073	CF8
2.76±0.024	2.70±0.035	2.86±0.050	2.81±0.062	CF9
2.99±0.045	3.08±0.055	3.04±0.045	3.11±0.052	CF10

Table 6: Tensile Strength Parameters of Blank Composite and Selected Drug Loaded Films

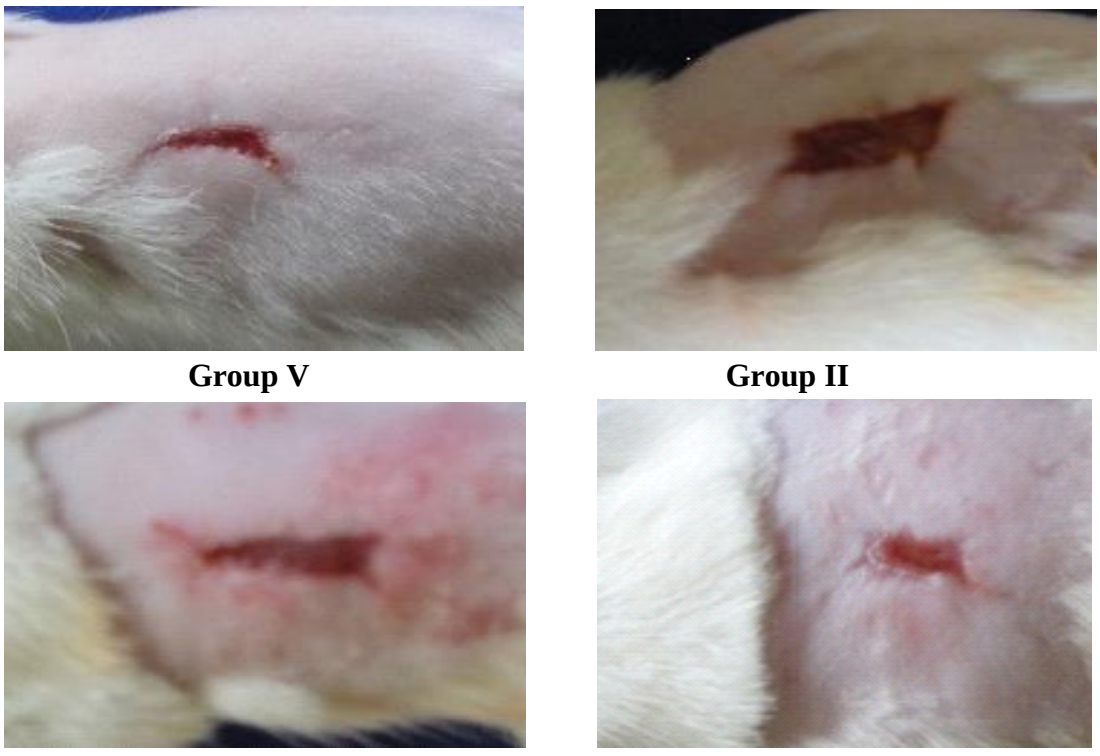
CF10	F6	F5	F4	F3	Tensile strength parameters
24.97	22.71	33.29	43.64	17.80	Maximum load (N)
1.74	2.67	1.12	1.17	0.67	Maximum extension (mm)
3.48	5.33	2.25	2.33	2.22	Elongation at break (%)
36.79	45.42	41.26	39.68	11.13	Tensile strength (mpa)

Table 7: Cumulative Percentage Drug Release of Selected Curcuma Longa Loaded Film (CF10).

Drug release(%) (Mean±S.D.)	Time (min)
0	0
15.93±1.37	10
31.18±1.23	20
39.37±1.11	30
49.17±1.04	40
59.96±0.86	50
70.15±0.78	60
79.37±0.83	70
88.86±0.64	80
97.95±0.18	90

Table 8: Percentage of Wound Contraction at Different Time Intervals in Different Groups

Wound contraction (%) (Mean±S.D.)				Group
28th day	**21 th day**	**14 th day**	**7th day**	
92.46±1.11	75.91±2.10	59.80±2.95	21.31±4.20	II (Untreated)
95.49±0.29	84.75±1.06	68.29±1.49	40.64±4.12	III (F1 treated)
98.78±0.10	90.74±0.69	76.72±1.33	54.33±3.66	IV (F6 treated)
99.52±0.11	94.86±0.45	83.41±2.24	59.93±2.79	V(CF10treated)



Group V **Group II**

Group IV **Group III**

Fig. 1: Comparison of Photographs of Wound on 14th Day In Different Groups

References

1) Lin SY, Chen K S, and Run-Chu L. *J. Biomaterials.* **(2001)**, 22, 2999–3004.

2) Purna S K, and Babu M. *J. Burns.* **(2000)**, 26, 54–62.

3) Winter G D. *J. Nature.* **(1962)**, 193, 293–294.

4) Kannon GA and Garrett A B**.** *J. Dermatol Surg*. **(1995)**, 21,583–590.

5) Mehrdad K, Mohammad S,and Zuhair M H. *European Polymer Journal*. **(2007),** 43,773–781.

6) Shelma R, Willi P, and Sharma CF. *Trends in Biomaterials and Artificial organs.* **(2008),** 22 : 107-111.

7) In-Yong K, Seog-Jin S, Hyun-Seuk M, and Mi-Kyong Y. *Biotechnology Advances.* **.(2008),** 26,1-21.

8) Emir B D and Raphael M O. *Journal of Bioactive and Compatible polymers.* **(2006),** 21**,** 351-368.

9) Shi C, Zhu Y, Ran X, Wang M, Su Y, and Cheng T. *Journal of Surgical research.* **(2009),** 133**,**185-192.

10) Daniela E and Camelia E O. *Chemical Engineering Communications*. **(2008)**, 1269-1291.

11) Koide S S. *Nutrition Research***.(1998)**,18, 1091-1101.

12) Hima B, Vidyavathi M, Kavitha K, and Sastry T P**.** *International journal of applied biology and pharmaceutical technology.* **(2011)**, 2,(1).

13) Harborne J B, Mabray T J, and Mabray H. **Physiology and Function of Flavonoi**..The flavoids. Acad. Press, New York, San Francisco.**(1975),** 970-1042.

14) Felicity ،B.; Clifford ،L.; Michael، A.; Oghenekome ،O. *J American-Eurasian Agric & Env. Sci***. (2007)**, 2, 103-111.

15) Tanwar YS. *The Dhaka University journal of Pharmaceutical Sciences* **. (2005),** 3, 25-37.

16) Nagwa F, Hanan E, and Mina T**. Implantable Biodegradable Sponges: Effect of Inter polymer Complex formation of Chitosan-gelatin on the release Behaviour of Tramadol HCl**. 33th Edition. D Dev and Ind Pharm**. (2007),** 7-17.