Bull. Pharm. Sci., Assiut University, Vol. 30, Part 2, 2007, pp. 111-129.

FORMULATION AND CHARACTERIZATION OF BIODEGRADABLE CHITOSAN FILMS FOR TOPICAL APPLICATION OF TERBINAFINE HCl

S. M. Ahmed[1], M. A. Ibrahim[2], H. A. Sarhan[3] and M. A. Amin[2]

[1]*Department of Industrial Pharmacy, Faculty of Pharmacy, Assiut University, Assiut, Egypt*
[2]*Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, El-Azhar University, Assiut branch, Egypt*
[3]*Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, El-Minia University, El-Minia, Egypt*

تهدف الدراسة الى امكانية تحضير اغشية عديدة الجزيئات مناسبة من الكيتوزان (منخفض الوزن الجزيئي بدرجة ازالة استلة ٨٠ ٨٥ %) كانظمة ايصال دوائية لعقار هيدروكلوريد تربينافين الذي يستخدم كمضاد للفطريات. تم دراسة تاثير تركيز العقار وطبيعة المواد الملدنة على الانطلاق المعملي للعقار وقد دلت النتائج على ان الاغشية المحضرة من الكيتوزان والمحتوية على المواد الملدنة قد اثرت بشكل ملحوظ على معدلات الانطلاق المعملى للعقار وان الانطلاق المعملى للعقار يتبع معادلة هيجوشى للانتشار. كما اوضحت الدراسة ايضا ان معدل الانطلاق المعملى للعقار يتناسب طرديا مع تركيز العقار في الاغشية. كما دلت الدراسة ايضا ان اضافة المواد الملدنة للاغشية كان له اثر كبير في زيادة معدلات الانطلاق المعملى للعقار من اغشية الكيتوزان ، وقد امكن ترتيب المواد الملدنة حسب قوة تاثيرها على معدلات الانطلاق المعملى كالاتي: ثلاثي خلات الجليسرول > عديد ايثيلين الجليكول > بروبيلين جليكول> ثلاثي بيوتيرات الجليسرول > ثلاثي ميثيل فثالات > ثلاثي ايثيل فثالات. كما تناول البحث دراسة الخواص الفيزوكيميائية لاغشية الكيتوزان المحتوية على هيدروكلوريد تربينافين باستخدام الاشعة تحت الحمراء ، المسح الحراري التفاضلي وحيود الاشعة السينية. وقد اثبتت الدراسة ان العقار قد تحول من الشكل البلوري الى الشكل اللابلوري وانه لاتوجد تفاعلات بين هيدروكلوريد تربينافين والكيتوزان. ايضا تم دراسة تاثير الاغشية البوليمرية المحتوية على العقار كمضاد للفطريات ووجد ان التركيز الادنى لتثبيط الفطر هو ١,٤ ميكروجرام/ مل وان قطر منطقة التثبيط للعقار من الاغشية كان اعلى من مثيله باستخدام الشاش المحتوي على نفس التركيزات من العقار. كما اوضحت الدراسة ان النشاط ضد الفطر قد ازداد من الاغشية المحتوية على ثلاثي خلات الجليسرول كمادة ملدنة.

Received in 14/7/2007, Received in revised form in 20/12/2007 & Accepted in 23/12/2007

Chitosan biodegradable films containing terbinafine HCl (Tr.HCl) were evaluated for their potential drug delivery at a controlled rate. Terbinafine HCl could be loaded at 1.8% w/w of polymer in films, which were translucent and flexible. The effect of drug loading and nature of plasticizers on the in-vitro release of Tr.HCl have been examined. Physicochemical characterization of Tr.HCl via thermal, spectroscopic, X-ray diffraction, and scanning electron microscopy techniques revealed information on the solid-state properties of Tr.HCl as well as chitosan in films. While chitosan was in an amorphous form, Tr.HCl seemed to be present in crystalline form in the films. It was found that the release rate of the drug was directly proportional to drug concentraton. Also medicated chitosan films plasticized with water- soluble plasticizers as glycerol triacetate (GTA), propylene glycol (PG), and polyethylene glycol 400 (PEG 400), produced fast release in comparison with water insoluble plasticizers as glycerol tributyrate (GTB), dimethyl phthalate (DMPH), and diethyl phthalate (DEPH). The characterizations of chitosan films conducted by IR, X-ray, and DSC, showed that no interaction occurred between Tr.HCl and chitosan polymer. The minimum inhibitory concentration (MIC) of the drug against candida albicans was investigated. Results showed that MIC of Tr.HCl was 1.4 µg/ml. The inhibition zone diameter of Tr.HCl chitosan films was higher than that of Tr.HCl normal dressing. Also antifungal activity of Tr.HCl was enhanced in plasticized chitosan films. The results were promising for topical formulation of Tr.HCl in biodegradable chitosan films and have the potential to be used as a novel drug delivery.

INTRODUCTION

In recent years, biodegradable polymeric system have gained importance for design of surgical devices, artifical organs, drug delivery systems with different routs of administrations, carriers of immobilized enzymes and cells, biosensors, ocular inserts, and materials for orthopedic applications[1]. These polymers are classified as either synthetic (polyesters, polyamides, polyanhydries) or natural (polyaminoacides, polysaccharides)[2]. Polysaccharides-based polymers represent a major class of materials, which includes agarose, aligante, carageenan and chitosan. Chitosan, β (1,4) 2-amino-2-D-glucose, is a cationic biopolymer produced by alkaline N-deacetylation of chitin, which is the main component of the shells of crab, shrimp, and krill. Chitosan has found many applications, including tissue engineering, owing to its biocompatibility, low toxicity, and

degradation in the body by enzymes such as chitosanase and lysozymes[3], which has opened up avenues for modulating drug release *in-vivo* in the treatment of various diseases. These chitosan-based delivery systems range from microparticles to nanoparticles[4], gels[5] and films[6]. Further, gels and films of chitosan have been used for oral delivery of chlorhexidine digluconate in the treatment of fungal infections[7]. In addition, chitosan has been extensively evaluated as a carrier of antineoplastic agents such as 5-flurouracial[8], mitoxantrone[9], cytarabine[10] and placlitaxel[11].

The film forming property of chitosan has found many applications in tissue engineering and drug delivery by virtue of its mechanical strength and rather slow biodegradation[12]. Some drug-loaded chitosan films are emerging as novel drug delivery systems[13&14], and films appear to have potential topical delivery of antifungal drugs.

Terbinafine HCl is a synthetic allylamine antifungal agent[15]. It can be administered orally as well as topical application. It is considered the agent of choice for treatment of dermatophyte nail infections[15]. The objective of this study was to develop a chitosan film loaded with certain concentrations of terbinafine HCl. These films have been evaluated for the release of impregnated terbinafine HCl, characterized by various physical techniques and microscopy, and examined for antifungal activity by zone of inhibition technique.

MATERIALS AND METHODS

Materials

Chitosan grades L*, degree of deacetylation (%DD) 80-85%, was purchased from Sigma-Aldrich company, Germany, Terbinafine HCl was gift sample from Novartias company (Cairo, Egypt), Absolute ethanol was purchased from March KgaA (Darmstadt, Germany), Acetic acid, potassium dihydrogen phosphate, sodium hydroxide, anhydrous calcium chloride, ammonium chloride and PEG 400 was obtained from El-Nasr Pharmaceutical Chemicals Co., (Egypt), Glycerol triacetate (GTA), Glycerol tributyrate (GTB), Diethyl phthalate (DEPH) and Dimethyl phthalate (DMPH) was obtained from Fluka Co., Germany, Silicon adhesive [Super Automotive and consumer products Co., (U.S.A.) Other solvents and chemicals were of analytical grade.

Equipment

Digital micrometer (Mitutoyo, Kanagawa, Japan); dissolution-test apparatus, SR11 6-flasks (Hanson research Co., USA), double beam spectrophotometer (Shimadzu, UV-150-02, Shimadzu Co., Japan); pH meter (Ama digital, Ama Co., Germany); IR–Spectrophotometer, [Shimadzu Co., Japan]; Differential Scanning Calorimeter, DSC-50 equipped with computerized data station (Shimadzu Co., Japan); X-ray diffractometer [Phillips Co., Netherlands]., which is equipped with curved graphite crystal mono-

chromater, automatic divergence slite and automatic controller PW/1710. The target used was CuK α radiation operating at 40 Kv and 30 mA (λ Ka= 1.5418 A). The system was calibrated using silicon disc and/or powder (d_{111}= 3.1355 a) as an external standard. The diffraction pattern was achieved using continuous scan mode with 2θ° ranging from 40° to 60°. JEOL, scanning electron microscope [JSM–5200, Japan].

Methods

Film preparation

The films were prepared using teflon plates as casting substrate (8 cm in diameter) by a casting solvent evaporation technique as follows: 2.5 g of chitosan was dissolved in 25 ml aqueous acetic acid solution (1% v/v) with a constant stirring for 48 hrs to give chitosan solution. Different concentrations of terbinafine HCl (1, 1.2, 1.4, 1.6 and 1.8% w/w of polymer) with or without different types of plasticizers at 20% w/w of polymer (GTA, PG, PEG400, GTB, DMPH, and DEPH). The resultant solution was left to stand until all air bubbles disappeared, then 8.5 ml of the bubble free liquid was poured into a circular teflon mold on dust-free-leveled surface, and left to dry at room temperature for 24 hrs.The dried films were evaluated within one week after their preparation[16].

Content uniformity of the films

To ensure uniform distribution of Tr.HCl in the prepared films, a content uniformity test was performed in triplicate. Samples representing different regions within the film were cut and weighted, and terbinafine HCl was extracted with 1:1 solvent mixture of acetonitrile and ethanol (v/v) twice for 12 hrs at room temperature. The extracts were collected and absorbance was measured at 265 nm against suitable blank, and the drug concentration was calculated.

Film thickness

The film thickness was determined at ten points of the film, using digital micrometer (Mitutoyo, Kanagawa, Japan), and the film thickness was recorded and found to be 22±0.2 μm.

***In–vitro* release studies**

The release experiment was performed according to paddle method (JP x II) at a rotational speed of 50 rpm, which was the optimum speed to prevent film rupture. The release medium was 400 ml of phosphate buffer (pH= 6.8) equilibrated at 37°C ± 0.5°C. It was taken into consideration that the used buffer volume kept sink conditions during the experiment. To avoid water evaporation, the vessels were covered with an aluminum foil during the experiments. The film was carefully pressed into the bottom of the glass vessel by the aid of silicon adhesive. At time intervals 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0 and 6.0 hrs, 5 ml sample was withdrawn and replaced by an equal volume of fresh

release medium previously equilibrated at 37°C. The amount of drug released (mg) was measured spectrophoto-metrically at λ_{max} 265 nm and plotted as a function of time.

A cumulative correction was made for the previously removed samples in determining the total amount released according to the following formula[17]:

$$C_n = C_n\ meas + 5/400\ \Sigma C_s^{n-1}\ meas$$

Where:

C_n *meas* = spectrophotometrically measured concentration,

C_n = concentration of the n^{th} sampling expected in the receiving medium if previous samples had not been removed,

n-1 = total volume of all samples removed prior to a sample being measured, and

Cs = total of all spectrophoto-metrically measured concentrat-ions at n-1 samples.

Solid-state characterizations

To study the molecular properties of terbinafine HCl and chitosan, the solid-state characterization was done by the application of thermal, infrared, and X-ray diffraction. During these studies, solid-sate characteristics of the drug and chitosan were compared with those of the film to reveal any changes occurring as a result of film preparation.

Infrared spectroscopy (IR)

Infrared spectroscopy spectra of untreated Tr.HCl, the polymer (chitosan L*; %DD 80-85), casted film of the drug with the polymer and its corresponding physical mixture were done by using at a range of 4000-400 cm^{-2}. Potassium bromide (KBr) disk method was used for powder samples. The samples were ground, mixed thoroughly with KBr and compressed at a pressure of 6 ton/cm^2 using IR compression machine. Chitosan films were directly used during the test.

Differential scanning calorimetry (DSC)

Differential Scanning Calorimetry thermograms were carried out on untreated Tr.HCl, the polymer (chitosan L*; %DD 80-85), casted film of the drug with the polymer and its corresponding physical mixture The procedure involved heating an accurately weighed sample (5 mg) encapsulated in an aluminum pan at a predetermined scanning rate (10°C/min) and over a predetermined temperature range 30°C to 400°C in the presence of nitrogen flow at a rate of 40 ml/min. Indium was checked by running the sample in triplicate, the standard deviations calculated were found negligible.

X-ray diffraction

The X-ray diffraction patterns of untreated Tr.HCl, the polymer (chitosan L*; %DD 80-85), casted film of the drug with the polymer and its corresponding physical mixture.

Scanning electron microscopic studies

The surface morphology of the drug-dispersed films before and after

drug release studies were examined. The test sample was attached to the metal stubs with double pressure sensitive adhesive tape and coated with thin layer of platinum to improve the conductivity. Scanning electron photomicrographs were taken at 15000-x magnification.

Culture and *in-vitro* susceptibility test

Determination of minimum inhibitory concentration (MIC) was performed by using the tube dilution technique as described by Shadomy and Espinel–Ingroff[18]. On the other hand, the sizes of the zones of inhibition were measured using the diffusion technique as described by Lorain[19].

Candida albicans was grown on sabaroud dextrose at 37°C for 24 hrs. This microorganism was inoculated onto the medium to give approximately $l0^6$ cells/ml for microorganism. The prepared suspension was diluted with sterile saline solution and adjusted to pH 6.5. Media growth is recorded after 24 hrs incubation at 37°C.

The lowest concentration of the drug in μg/ml that prevents *in-vitro* growth was taken as the MIC. A control was done in parallel with the test for this sample. The mean of at least 3 readings was determined.

The prepared Tr.HCl: chitosan L* films containing different drug concentrations (1, 1.2, 1.4, 1.6, 1.8% Tr.HCl and 1.8. Tr.HCl% w/w of polymer in the presence of GTA) was tested for antifungal activity against c*andida albicans*. Also Tr.HCl normal dressing were prepared and subjected to the same test.

For inoculation of nutrient broth, a volume of 0.1 ml of culture was placed onto the surface of sabaroud dextrose plate. Discs of Tr.HCl: chitosan L* (different concentrations) films and normal dressing containing 1.8% w/w of terbinafine HCl were placed and gently pressed down on the surface of sabaroud dextrose fluid media. Plates were incubated for 24-48 hrs at 37°C and zones of inhibition were measured.

RESULTS AND DISCUSSIONS

Content uniformity

Terbinafine HCl was extracted from different regions of chitosan film using Acetonitrile : ETOH (1:1) solvent system. After normalization of the amount of terbinafine HCl on weight basis of film, the results indicated that the variation in distribution of terbinafine HCl in different regions of film was <13%.

Effect of drug loading on the release pattern from chitosan films

The effect of different concentrations of terbinafine HCl (1%, 1.2%, 1.4%, 1.6% and 1.8% w/w of polymer) on the release rate of the drug from chitosan films was investigated.

The percentage of drug concentration were corresponding to 25, 30, 35, 40 and 45 mg/film drug for the volume of the dissolution medium (phosphate buffer of pH= 6.8) was adjusted for each film to

afford sink conditions and to obtain spectrophotometrically measurable sample for low drug concentration. The obtained results are listed in Tables 1 & 2 and Fig. 1.

The release rate constant of the drug increased as the concentration in the film increased. Straight line of high correlation coefficient (r= 0.999) was also obtained when the amount of the drug released was plotted against the square root of time (Fig. 1).

The release data of terbinafine HCl from chitosan films were analyzed according to zero-order, first order[20], and diffusion controlled release mechanisms according to the simplified Higuchi model[21]. The obtained results proved that the gradual increase in the drug loading caused a significant increase in the value of $t_{1/2}$ and the release of the drug from chitosan films followed Higuchi diffusion model (Tables 1 & 2).

Kanke *et al.*[22] reported that predinsolone was released from chitosan film following zero order wheras; Chandy, and Sharms[23] reported first order kinetics. Puttipipatkhachorn *et al.*[24] found that the release of salicylic acid from chitosan films obeyed Fickian diffusion control mechanism with subsequent zero-order.

Thacharodi and Panduranga Rao studied the diffusion behavior of some drugs through chitosan membranes; they evaluated the diffusion efficacy of chitosan films for both hydrophilic and hydrophobic drugs[25-27]. The water-soluble drug (propranolol HCl) could be transported through films via pore mechanism[27]; wheras the hydrophobic drug (nifedipine) could be influenced by both partition and pore mechanisms operating concurrently[26&27].

The increase in the release rate constant of a given drug increases upon increasing drug concentration in the film could be explained by assuming that matrix porosity necessary for the diffusion pathway may create pores by the dispersed drug. Increasing drug concentration in the film may result in increasing the degree of internal porosity[28]. Similar results were obtained in this study, where the release of Tr.HCl from chitosan films was found to be through pores. Scanning electron microscopy photographs of the films further confirmed this after the drug release (Fig. 2).

Effect of inclusion of different types of plasticizers on drug release pattern

Five chitosan L* films containing different plasticizers were prepared to investigate the effect of plasticizer nature on Tr.HCl release. The concentration of each plasticizer was 20% w/w of polymer and each film containing 45 mg Tr.HCl (1.8% w/w of polymer). The obtained results were listed in Tables 3 & 4 and graphically represented in Figure 3**.**

When the medicated plasticized chitosan films were immersed in phosphate buffer (pH= 6.8), two factors could be taken into

Table 1: Kinetic data of the drug released from chitosan L* films at different concentrations of terbinafine HCl.

Mechanism of Release		Drug concentration % w/w of polymer				
		1	1.2	1.4	1.6	1.8
First order	r	0.997	0.994	0.987	0.995	0.987
	K_0 (min^{-1})	0.0009	0.0010	0.00093	0.0094	0.00088
Zero order	r	0.991	0.989	0.977	0.990	0.987
	K_1 (mg/min.)	0.0227	0.028	0.034	0.043	0.0507
Higuchi diffusion	r	0.999	0.999	0.995	0.998	0.998
	K_h ($mg/cm^2/min^{1/2}$)	0.617	0.785	0.940	1.178	1.383
Log Q vs log t	r	0.998	0.999	0.994	0.998	0.998
	Slope	0.670	0.545	0.564	0.556	0.560
Best fit model		Higuchi	Higuchi	Higuchi	Higuchi	Higuchi

r : Correlation coefficient
K_0 :Zero order release rate constant
K_1 : First order release rate constant
K_h : Diffusion release rate constant

Table 2: Effect of drug concentration on release rate constant (k), half-life ($t_{1/2}$) for chitosan L* films containing different concentrations of terbinafine HCl according to Higuchi-diffusion model.

Drug concentration (% w/w of polymer)	Release rate constant (k)	Correlation coefficient (r)	$t_{1/2}$ (min.)
1	0.617	0.999	410.44
1.2	0.785	0.999	365.12
1.4	0.940	0.995	452.69
1.6	1.178	0.998	450.39
1.8	1.383	0.998	470.54

Table 3: Kinetic data of the drug released from chitosan L* films at different types of plasticizers, each films containing 45 mg terbinafine HCl.

Mechanism of Release		Plasticizers types					
		GTA	PEG400	PG	GTB	DMPH	DEPH
First order	R	0.992	0.994	0.999	0.995	0.818	0.995
	K_0 (min^{-1})	0.002	0.001	0.006	0.002	0.001	0.001
Zero order	R	0.989	0.990	0.998	0.992	0.991	0.993
	K_1 (mg/min.)	0.0254	0.025	0.021	0.021	0.0197	0.0192
Higuchi diffusion	R	0.998	0.999	0.995	0.999	0.999	0.998
	K_h ($mg/cm^2/min^{1/2}$)	0.690	0.666	0.541	0.588	5.35	0.514
Log Q vs log t	r	0.999	0.999	0.994	0.999	0.999	0.999
	Slope	0.512	0.534	0.432	0.524	0.528	0.528
Best fit model		Higuchi	Higuchi	Higuchi	Higuchi	Higuchi	Higuchi

r : Correlation coefficient

K_0 :Zero order release rate constant

K_1 : first order release rate constant

K_h : Diffusion release rate constant

Table 4: Effect of different plasticizers on release rate constant (k), half-life ($t_{½}$) for chitosan L* films each containing 45 mg terbinafine HCl according to Higuchi-diffusion model.

Plasticizers	Release rate constant (k) ($mg/cm^2/min^{1/2}$)	Correlation coefficient (r)	$t_{½}$ (min.)
Plain	1.0367	0.998	470.89
GTA	0.6906	0.998	1063.56
PEG400	0.668	0.999	1134.63
PG	0.541	0.995	1729.29
GTB	0.534	0.999	1775.75
DEPH	0.525	0.999	1836.42
DMPH	0.519	0.998	1879.33

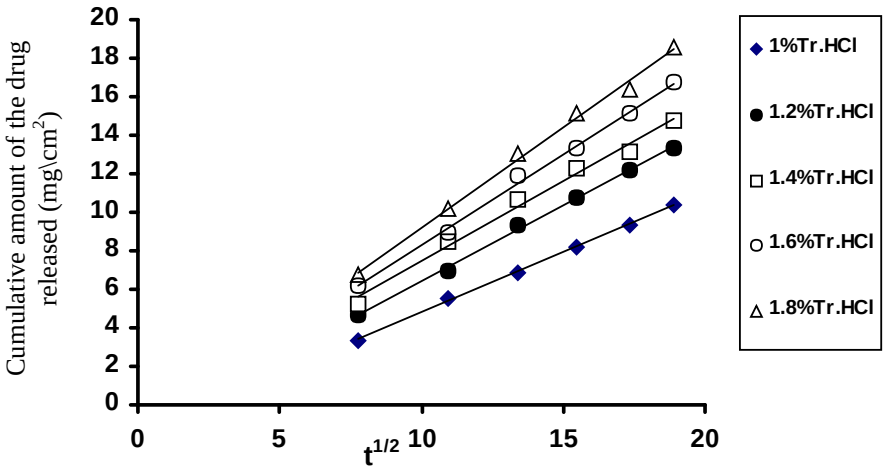

Fig. 1: Amount of drug released mg/cm^2 from chitosan L*films at different concentrations of terbinafine hydrochloride.

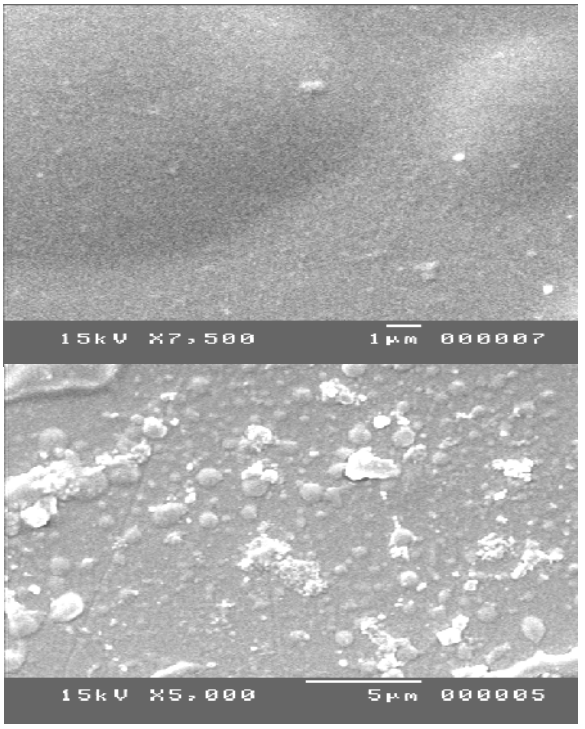

Fig. 2: Scanning electron micrographs of films containing 1.8% w/w terbinafine hydrochloride. **A:** Before release of the drug, **b:** After release of the drug.

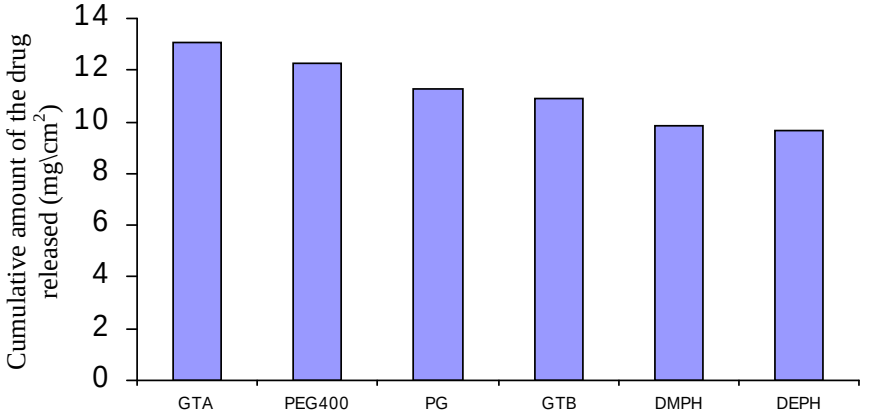

Fig. 3: Comparison of drug release profile for chitosan L* films each containing 45 mg terbinafine hydrochloride and plasticized with 20% w/w of polymer with different plasticizers after 6 hours.

consideration to explain the effect of plasticizer content on drug release profile. The first is the solubility of the plasticizer in water (i.e. the probability of hydrogen bonding between the plasticizer and water molecules), while the second is the extent of channels or pathways through which the plasticizer will be leached throughout the polymeric matrix[29].

The obtained results showed that the effect of different plasticizers on the release rate of the drug from chitosan films can be arranged in the following order: GTA > PEG400 > PG > GTB > DMPH > DEPH.

It is concluded that GTA, PEG400 and PG could be leached through a continuous hydrated capillary network of channels, which is a major characteristic feature for all tested water –soluble plasticizers. Also the hydrophilic nature or the solubility of the plasticizers in the release medium can be considered as an important factor in controlling this process. Dimethyl phthalate (DMPH), diethyl phthalate (DEPH), and glycerol tributyrate (GTB) were investigated in this study as examples for water-insoluble plasticizers. Upon diffusion of the film, Tr.HCl would diffuse through the hydrated voids created by those water-insoluble plasticizers. This may explain the release of small amount of Tr.HCl from chitosan films plasticized with water-insoluble plasticizers compared to those plasticized with water-soluble plasticizers.

IR spectroscopy

Infrared spectra of chitosan powder, terbinafine HCl, and films were recorded to get information about interactions between the drug and the polymeric carriers in the solid state. The transmission spectra of chitosan powder exhibited broad peaks in the range from 3520 to 3445 cm^{-1} (Fig. 4a), which were assigned to OH stretching, indicating inter-molecular hydrogen bonding of chitosan molecules. An overlap was seen in the same region of NH stretching. The C=O stretching (amid I) peak near 1650 cm^{-1} and NH bending (amid II) peak near 1556 cm^{-1} regions were observed in the spectra of the chitosan grades L* representing the structure of N–acetyl glucose amine[30].

The principal peaks of terbinafine HCl appear at 3300 cm^{-1} ($N–CH_3$) (Fig. 4b). The peak remained visible upon combination with the polymer and showed no discernable shifts or broading. Thus, from IR spectral analysis, there is no change in the location or width of the infrared absorption bands of the drug and chitosan either in the physical mixture or in the medicated chitosan films. Therefore, it might be concluded that there was no interactions between the drug and the polymer used.

Differential scanning calorimetry (DSC)

In order to shed some light on the possibility of solid state changes, DSC was carried out for the untreated drug, chitosan L*, 80-85% DD alone, drug: chitosan L* physical mixtures,

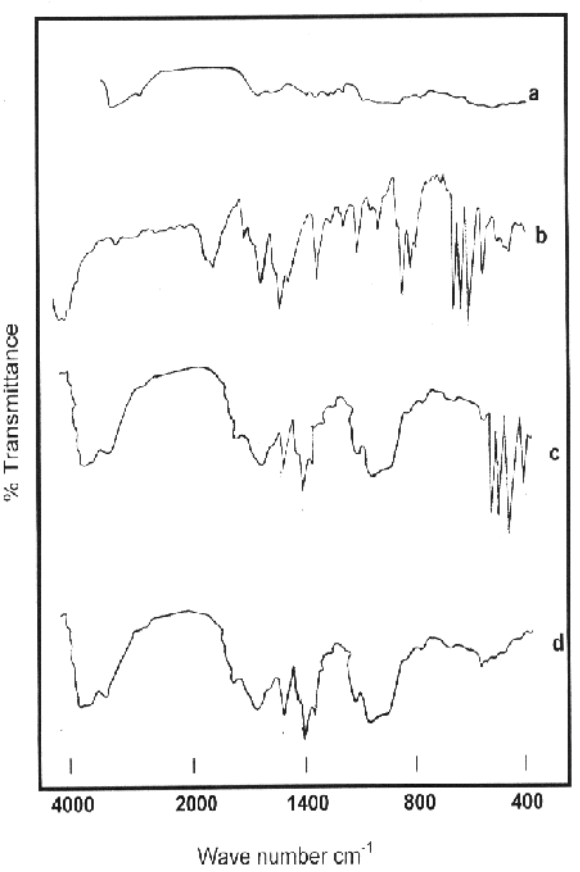

Fig. 4: IR spectra of **a:** chitosan L* powder, **b:** drug alone powder, **c:** physical mixture of Tr.HCl-chitosan L* and **d:** cast film of Tr.HCl-chitosan L*.

and the corresponding medicated films.

Figure 5b shows the DSC curve of terbinafine HCl that shows a single melting endothermic peak, melting point at 176.54°C and ΔH= -28.64 Kcal/Kg.

Chitosan L*; 80-85% DD free film, as reported in the literature[31], show no melting endothermic peaks. The thermograms of physical mixtures show the same drug characteristic with minor changes.

Differential Scanning Calorimetry scans of the drug incorporated into the polymer showed no characteristic peaks of the drug. The disappearance of the drug peaks at 1.8% w/w of polymer Tr.HCl polymeric film is related to the drug in the cast films present in the amorphous form or in the extremely fine crystallites of molecular dispersion, which could not

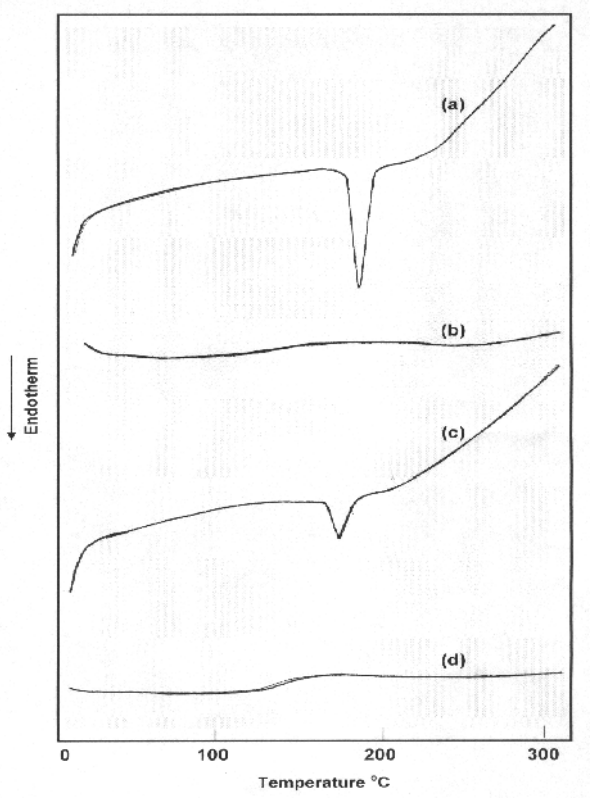

Fig. 5: DSC thermograms of **a:** drug alone, **b:** chitosan L* powder, **c:** physical mixture of Tr.HCl-chitosan L* and **d:** cast film of Tr.HCl-chitosan L*.

detected by DSC. Similar findings were obtained by Kodha *et al.*[32] who noticed the disappearance of endothermic peak of Lidocaine HCl in the solid dispersion films containing hydroxy propyl cellulose (HPC). They explained this phenomenon on the bases that complete transformation of the drug from crystalline to amorphous form when incorporated into the polymer.

X–ray diffractometry

To get further evidence on the solid state changes, X-ray diffraction spectra were carried out for untreated drug, medicated chitosan L* films (1.8% w/w of polymer of Tr.HCl) and their physical mixtures as well as individual components.

The pattern of Tr.HCl (Fig. 6b) alone shows the presence of numerous distinct peaks indicating that, the drug is present as crystalline form with characteristic diffraction peaks appearing at 2θ of 6.81, 15.70, 20.29 and 25.57 degree, which were selected for comparative purposes.

On the other hand, chitosan L*, %DD 80-85 polymer didn't show any

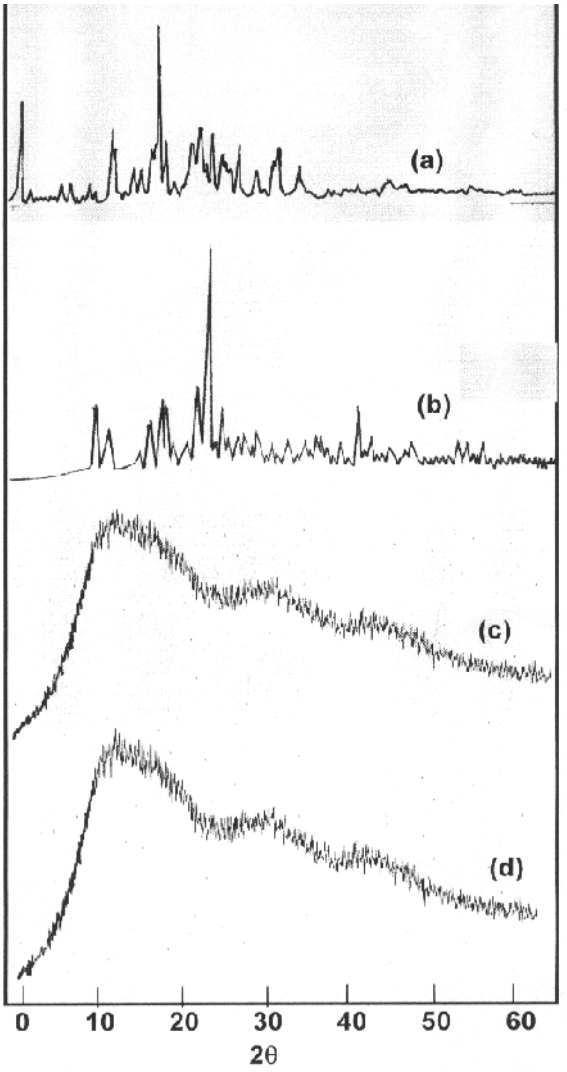

Fig. 6: X-ray pattern of **a:** drug alone, **b:** chitosan L* powder, **c:** physical mixture of Tr.HCl-chitosan L* and **d:** cast film of Tr.HCl-chitosan L*.

diffraction peaks indicating its amorphous nature (Fig. 6a). The diffraction patterns of cast films formed from Tr.HCl with chitosan L* polymer are characterized by complete absence of the drug characteristic peaks and conversion to the amorphous form. These findings comply with the data obtained by differential scanning calorimetry where the melting endothermic peaks of drug are absent in the cast film.

These results are in agreement with Kodha *et al.*[32] who reported the disappearance of X-ray diffraction peaks of drug crystals in the Lidocaine-EC-HPC solid dispersion film.

Antifungal activity of terbinafine HCl polymeric films

The minimum inhibitory concentration (MIC) of Tr.HCl against *candida albicans* is found to be 1.4 µg/ml. Thus, it can be concluded that this microorganisms was susceptible to Tr.HCl.

The antimicrobial effect of chitosan in the form of film is due to the structural modification of chitosan to chitosanium acetate resulting in a cross-linking between the polycation in chitosonium acetate and the anions on the surface of microorganisms which altered the membrane permeability, thereby resulting in a leakage of glucose and lactate dehydrognose from its cell. This is in agreement with earlier mechanism by Tasi and Su[33] underlying the inhibitory activity of shrimp chitosan (98% DD) against *Escherechia coli.*

Also, Senel *et al.*[7] founded that chitosan gel was less active than chlorhexidine gluconate solution against *candida albicans* and the highest antifungal activity obtained with 2% chitosan gel containing 0.1% chlorhexidine.

A Quantitative comparative study of the antifungal activity of medicated films with normal dressing, each containing the same concentrations of Tr.HCl, was performed and the results were shown in Table 5 and Fig. 7. It was found that medicated chitosan L* films showed a higher response in the inhibition zone than normal dressing.

The size of inhibition zones for medicated chitosan L* films at different drug concentrations showed a dramatic increase in the inhibition zone sizes with increasing drug concentration (Table 5).

The incorporation of (10% w/w of polymer) GTA into medicated chitosan L* films, resulted in higher response in the inhibition zone sizes for the drug. The inhibition zone sizes reflected quantitative concentration gradient established by diffusion of the drug through a given medium and the susceptibility of the tested microorganism (Fig. 8).

The obtained results revealed that the benefit of chitosan L* 80-85% DD as film former and delivery for antifungal drug, and it was recommended to use medicated chitosan L* films for topical treatment of fungal infections.

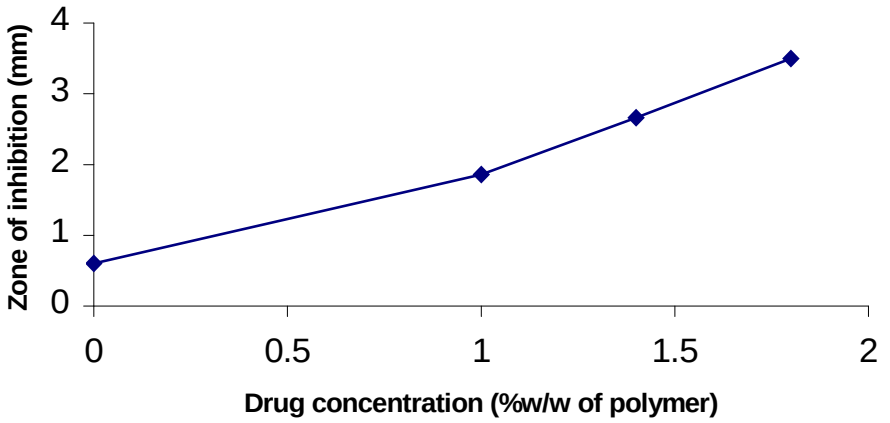

Fig. 7: Antifungal activity of medicated chitosan L* films at different drug concentrations.

Table 5: Antifungal efficacy of medicated chitosan L* films against *Candida Albican* at different drug concentrations.

Drug concentration	Zone of inhibition (diameter in cm)
Plain (o drug)	0.6
1	1.86
1.4	2.66
1.8	3.5
Normal dressing	3.25
Plasticized film	6.30

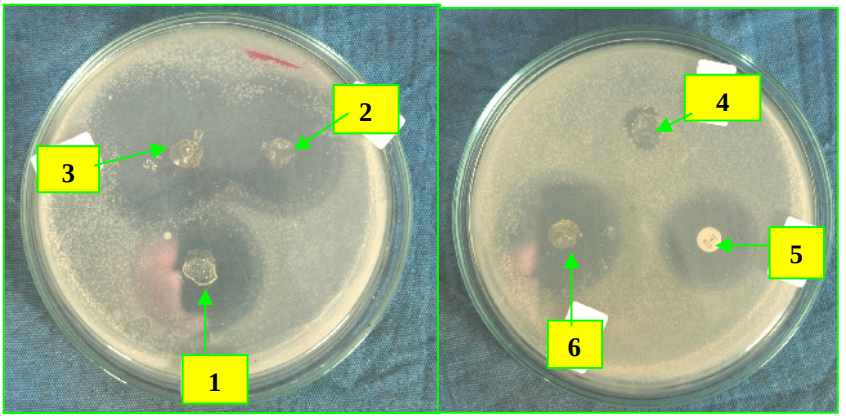

Fig. 8: Photographs represent the antifungal activity of different terbinafine.HCl formulations against *Candida albicans.*

1 10% w/v chitosan film containing 1% w/w terbinafine.HCl.
2 10% w/v chitosan film containing 1.4% w/w terbinafine.HCl.
3 10% w/v chitosan film containing 1.8% w/w terbinafine.HCl
4 Non-medicated chitosan film (10% w/w).
5 Normal dressing (1.8% w/w).
6 Plasticized chitosan film (1.8% w/w).

REFERENCES

1. N. Angeiova and D. H, Trends Biotechnol., 17, 409, 1999.
2. O. Pillai and R. Panchagnula, Curr. Opin. Chembiol., 5, 447, 2001.
3. Sh. Pangbum, PV. Trescony and G. Heller, Biomaterials, 3, 105, 1982.
4. I. B. Tsvetkova, L. M. Bronstein, S. N. Sidorov, O. L. Lependina, M. G. Sulman, P. M. Valetsky, B. Stein, L. Zh. Nikoshvili, V. G. Matveeva, A. I. Sidorov, B. B. Tikhonov, G. N. Demidenko, L. Kiwi-Minsker and E. M. Sulman, J. Molec. Cat., 276, 116, 2007.
5- N. Boucard, C. Viton, D. Agay, E. Mari, T. Roger, Y. Chancerelle and A. Domard, Biomaterials, 28, 3478, 2007.
6- P. C. Srinivasa, M. N. Ramesh and R. N. Tharanathan, Food Hydrocolloid, 21, 1113, 2007.
7- S. Senel, G. Ikinci and S. Kas, Int. J. Pharm., 193, 197, 2000.
8- J. Zan, D. Zhu, F. Tan, G. Jiang, Y. Lin and F. Ding, Chin. J. Chem. Engin., 14, 235, 2006.
9- Sr. Jameela and A. Jayakrinan, Biomaterials, 16,769, 1995.

10- Md. Blanco, C. Gomez and R. Olmo, Int. J. Pharm., 202, 29, 2000.
11- A. Miwa, A. Ishiba and M. Nakano, Pharm. Res., 15, 1844, 1998.
12- J. Ma, H. Wang and B. Ha, Biomaterials, 22, 331, 2001.
13- S. Miyazaki, H. Yamaguchi and M. Takada, Acta Pharm. Nord., 2, 401, 1990.
14- B. Anand Babu and P. Ramesh, J. Pharm. Sci., 6, 1208, 2004.
15- C. Dollery in "Theraputic Drugs", Churchill Livingstone, New York, 2nd Ed., 1999, 2, pp. t41-t44.
16- D. Thacharodi and K. Panduranga Rao, Int. J. Pharm., 96, 33, 1993.
17- J. Sciara, and S. P. Patel, J. Pharm. Sci., 10, 65, 1976.
18- S. Shadomy and A. Espinel-Inrraff. in: "Manual of Clinical Microbiology", Washington, D.C., U.S.A. 3rd Ed., 1980, pp. 647-653.
19- V. Lorain, in "Antibiotics in Laboratory Medicine", Baltimore USA 2nd Ed., 1986, p. 428.
20- A. Martin, J. Swarbrick, and A. Cammarata, in "Physical Pharmacy", Lea and Febiger, 2nd Ed., 1983, pp. 358-359.
21- W. I. Higuchi, J. Pharm. Sci., 51, 802, 1962.
22- M. Kanke, H. Katayama, S. Tsuzuki and H. Kuramoto, Chem. Pharm. Bull., 37, 523, 1989.
23- T. Chandy, and C. P. Sharma, Biomat. Art. Cells Immob. Biotech., 19, 745, 1991.
24- S. Puttipipatkhachorn, J. Nunthanid, K. Yamamoto, and G. E. Peck. J. Control. Release, 75, 143, 2001.
25- D. Thacharodi, and K. Panduranga Rao, J. Chem Tech. Biotechnol., 58, 177, 1993.
26- D. Thacharodi, and K. Panduranga Rao, Int. J. Pharm., 96, 33, 1993.
27- D. Thacharodi, and K. Panduranga Rao, Biomaterials, 17, 1307, 1996.
28- S. Shin, and S. Cho, Drug Dev. Ind. Pharm., 22, 299, 1996.
29- A. Ismail, M. G. Abd El Mohsen, and H. A. El-Ghamry, Zgazig J. Pharm. Sci., 7, 111, 1998.
30- T. Imai, S. Shiarishi, H. Saito and M. Otagiri, Int. J. Pharm., 67, 11, 1991.
31- J. Nunthanid, S. Puttipiptk-hachorn, K. Yamamoto, and G. E. Peck, Drug Dev. Ind. Pharm., 27, 143, 2001.
32- Y. Kodha, H. Kobayashi, Y. Baba and H. Yuasa, Int. J. Pharm., 158, 147, 1997.
33- G. J. Tsai. and W. H. Su, J. Food Port, 62, 239, 1999.