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Controlled release of antifungal Miconazole nitrate from crosslinked poly (vinyl alcohol) hydrogel

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ABSTRACT: Poly (vinyl alcohol) hydrogel was prepared by the reaction between 99% hydrolysed poly (vinyl alcohol) with gluteraldehyde as a crosslinking agent . The hydrogel was loaded with antifungal Miconazole nitrate . The swelling ratio of the polymer antifungal delivery system was determined in Simulated Gastric Fluid SGF , Simulated Intestinal Fluid SIF and distilled water . The release rate of Miconazole nitrate from hydrogel was studied by using U.V. technique at constant temperature (37 oC) in SGF and SIF . The mechanism of degradation for the polymer antifungal delivery was also determined by bulk erosion mechanism . Finally, the inhibition zone diameters for Miconazole nitrate , polymer miconazole nitrate delivery and polymer alone were studied against three types of fungi , Candida albicans , Cryptococcus neoformans and Fusarium oxysporum .

INTRODUCTION:

Hydrogels are three-dimensional crosslinked polymer structures which are able to swell in the aqueous environment . The capacity of super swelling hydrogels to absorb water is enormous and can be as much as 1000 times the mass of polymer. Hydrogels applications have been extensively studied because they are glassy in the dry state but swell to an elastic gel upon water penetration [1,2,3] .

Hydrogels are a class of biomaterials that receive increasing commercial attention. Hydrogel -based controlled drug delivery is among the current topics of intense interest. By using suitable polymeric materials to construct hydrogel delivery systems ,predictable release profiles of bioactive agents could be achieved over a period of time [4,5] .

Hydrogels can protect the drug from hostile environment ,e.g. the presence of enzymes and low pH in the stomach . Hydrogels can also control drug release by changing the gel structure in response to environmental stimuli [6] .

The permeability and release rate of drugs are influenced by the type of releasing agent and the water content in hydrogels . Despite the high water content (10 –95 %) of the hydrogels, the system may also be used for the release of drugs that are poorly soluble in water . Solute transport through a polymer membrane is either via the pore or partition mechanism . In the pore mechanism , the solute diffuses through the water filled pores while in the partition mechanism , the solute transport is presumed to occur by a process involving the dissolution of the solute within the polymer followed by the diffusion through the membrane [7] .There are two different modes for the erosion process of the polymer drug delivery systems :surface and bulk erosion . For surface eroding device , losing of the materials occurs at the surface only , leading to the decrement of the size . For bulk eroding device , the erosion would not be concentrated on the surface of the device , which would bring proper size of the device for considerable portion of time during application [8] . Poly (acrylamide / maleic acid) hydrogel system may be used as local therapeutic antifungal drugs [9] .

In this study ,the utility of polymer hydrogel prepared from poly (vinyl alcohol) which crosslinked with gluteraldehyde for the controlled release of miconazole nitrate, has been investigated.

MATERIALS AND METHODS :

Materials

Miconazole nitrate was supplied by (SDI Co.) , poly (vinyl alcohol) 99% , hydrolysis was supplied by (Aldrich Co.) , gluteraldehyde was supplied by (Fluka Co.) . All of the materials

were used as received . Fig. (1) shows the structure of miconazole nitrate .

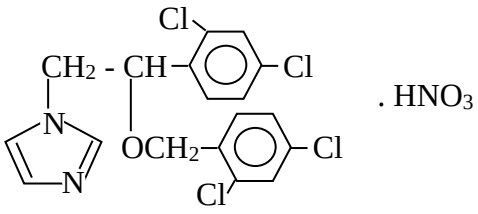

Fig.(1) : Structure of Miconazole nitrate

Preparation of the antifungal matrix:

10 g poly vinyl alcohol was dissolved in 100 ml distilled water at 60 ^{o}C . 0.5 g miconazole nitrate was dissolved in 40 ml absolute ethanol then 60 ml of distilled water was added . The solution of miconazole nitrate was added to poly (vinyl alcohol) solution with stirring until it becoms homogeneous , then 2 ml gluteraldehyde , 0.5 ml concentrated sulphuric acid were added . The mixture was heated at 60 ^{o}C with stirring . After one hour the mixture was cooled and coasted in Peter dish and placed in oven at 40 ^{o}C for two days .

***Releasing study*:**

The dry sheet of crosslinked poly (vinyl alcohol) miconazole nitrate delivery sample (8 mm diameter , 0.5 g weight) was immersed into SGF fluid (pH = 1.2) and SIF fluid (pH = 8.2) . The released rate of miconazole nitrate was followed by (Du 530/Beckman coolter) spectrometer at 271 nm .Fig.2 shows the release of miconazole nitrate at 37 ^{o}C in SGF and SIF fluids as a function of time .

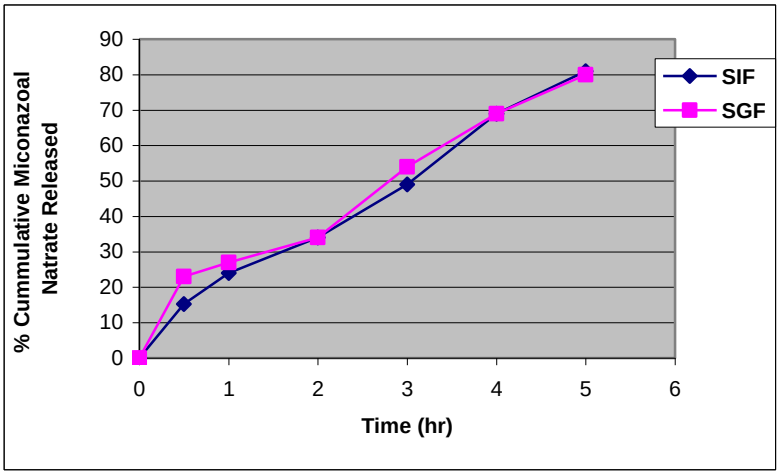

Fig.(2) : the release of miconazole nitrate at 37 ^{o}C in SGF and SIF fluids as a function of time .

The biological methodology:

Fungal isolates:

Three fungal isolates were tested for antifungal susceptibility which include two yeasts *Candida albicans* and *Cryptococcus neoformans* and one isolate of mold *Fusarium oxysporum* which all grow on SDA medium .

Fungal inocula :

A suspension for each isolate was prepared and adjusted to 10^{6} cell/ml by using haemocytometer [10] .

***Test media* :**

Emmons modification Sabouraud's dextrose agar (ESDA) (2% dextrose) was used for In Vitro susceptibility test . the pH of the medium was adjusted to 6.8 – 7.0 .

Drugs :

The antifungal drugs that were used to test their activity include Miconazole nitrate and the matrix of the antifungal drug with crosslinked polymer .

Antifungal susceptibility test :

This test was performed by agar diffusion method [11] , 0.2 ml of fungal inocula was placed on the surface of ESDA media and then spread with L – shape glass rod and the plate was left for 1 hr. . The antifungal drugs were placed in central pore which included , 0.5 gm of miconazole nitrate , 0.5g of miconazole nitrate with polymer , also used polymer alone (0.5 gm) as control plates for the polymer .

All isolates were grown on ESDA as a control . all plates were incubated at (25 ± 2 ^{o}C) . The inhibition zones of each isolate were recorded according to the growth of their controls . Duplicate plates were used for each test . The antifungal activity was given in tables (1 – 3) .

Table (1) : The inhibition zone diameter for miconazole nitrate and miconazole nitrate polymer delivery against the fungus *Candido albicans.*

Antifungal	Inhibition zone diameter (cm) after					
	3 days	4 days	5 days	6 days	7 days	10 days
Miconazole nitrate	3.0	3.0	2.8	2.7	2.7	2.7
Miconazole nitrate polymer delivery	5.0	4.8	4.8	4.8	4.8	4.8

Table (2) : The inhibition zone diameter for miconazole nitrate and miconazole nitrate polymer delivery against the fungus *Cryptococcus neoformans.*

Antifungal	Inhibition zone diameter (cm) after					
	3 days	4 days	5 days	6 days	7 days	10 days
Miconazole nitrate	4.7	4.7	4.5	4.4	4.4	4.4
Miconazole nitrate polymer delivery	5.0	5.0	4.9	4.7	4.7	4.7

Table (3) : The inhibition zone diameter for miconazole nitrate and miconazole nitrate polymer delivery against the fungus ***Fusarium oxysporum.***

Antifungal	Inhibition zone diameter (cm) after					
	3 days	4 days	5 days	6 days	7 days	10 days
Miconazole nitrate	0	0	0	0	0	0
Miconazole nitrate polymer delivery	9.0**(no growth)**	9.0**(no growth)**	9.0**(no growth)**	9.0**(no growth)**	9.0**(no growth)**	9.0**(no growth)**

***RESULTS AND DISCUSSION*:**

poly (vinyl alcohol) was crosslinked with gluteraldehyde by the formation of new acetal linkage between the chains to convert the water soluble poly (vinyl alcohol) to hydrogel . Miconazole nitrate has low solubility in water , The reform it was dissolved in absolute ethanol then water to be homogeneous with polymer solution then the crosslinking agent was added to prepare the hydrogel polymer antifungal delivery matrix . The swelling ratio (Q) for the polymeric matrix was determine in SGF and SIF fluid by the following equation :

Q = weight of swollen gel – weight of dried gel / weight of dried gel

The swelling ratio of polymeric matrix equals (0.46) in SGF fluid , (0.40) in SIF fluid and (0.32) in distilled water .

Fig. (2) shows the similar behaviour of the release rates of miconazole nitrate at 37 ºC in SGF and SIF fluids , this can be due to the mechanism of the release which is swelling followed by diffusion and the polymeric matrix has similar values of swelling ratio in SGF and SIF fluid. On the other hand , the mechanism of degradation was determined . Figures (3 and 4) show that the polymeric matrix system was degraded by bulk eroding mechanism [8, 12] .

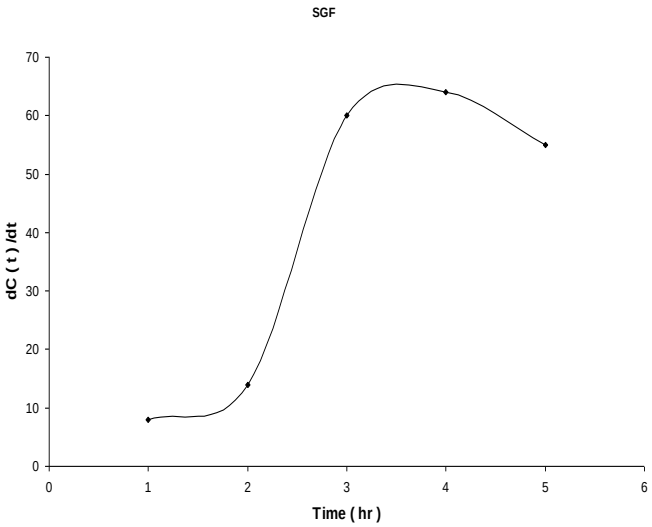

Fig. (3) : The degradation of polymer antifungal delivery in SGF at 37 ºC

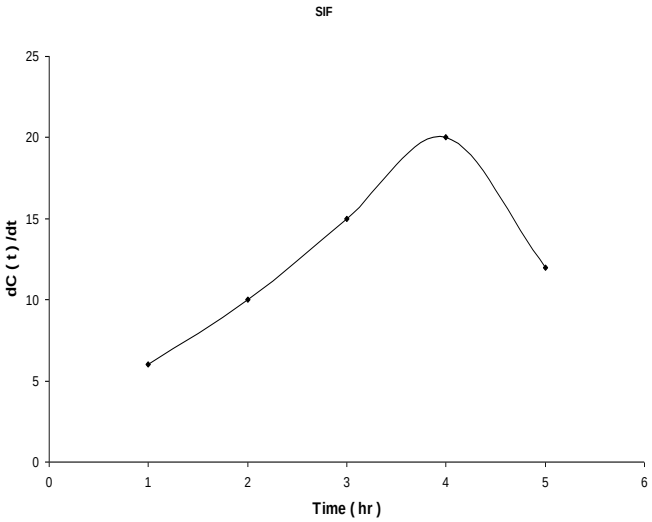

Fig. (4) : The degradation of polymer antifungal delivery in SIF at 37 ºC

The antifungal activity was given in tables (1 – 3) .The miconazole nitrate polymer showed more antifungal activity all isolates than miconazole nitrate alone .

The results showed that the Miconazole nitrate and polymer Miconazole nitrate delivery have different activity on the virulence of these isolates . Table (3) shows that Miconazole nitrate has no activity against *F. oxysporum* in comparison with its effect on *C. albicans* and *Cr. neoformans* , these results are related to the virulence of isolates and their ability to produce chlamydospoes which are resistant structures in unsuitable conditions . The complex of Miconazole nitrate has clear capability to defeat fungal defences by absolutely no growth of all isolates.

In addition , we studied polymer activity alone which showed that it has no activity against all isolates as shown in (Figs. 5 – 10) .

Fig.(5): the effect of miconazole nitrate and polymer miconazole delivery on the fungal *Cryptococcus neoformans.*

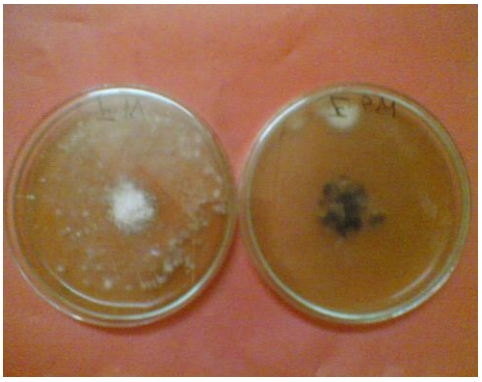

Fig.(6): the effect of miconazole nitrate and polymer miconazole delivery on the fungal *Fusarium oxysporum.*

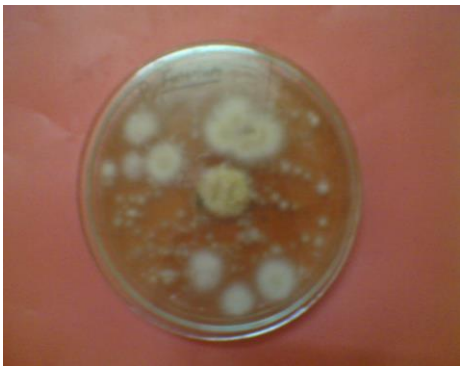

Fig.(7): the effect of polymer on the fungal *Fusarium oxysporum*.

Fig.(8): the effect of polymer on the fungal *Cryptococcus neoformans.*

Fig.(9): the effect of polymer miconazole delivery on the fungal *Candido albicans.*

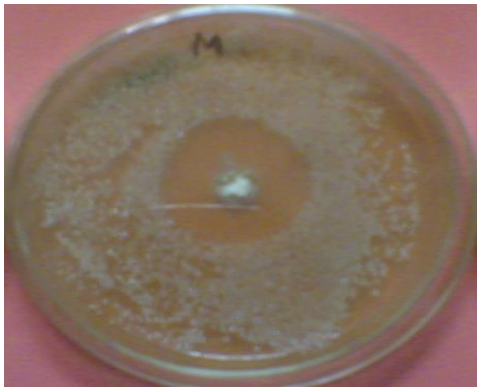

Fig.(10): the effect of miconazole nitrate On the fungal *Candido albicans.*

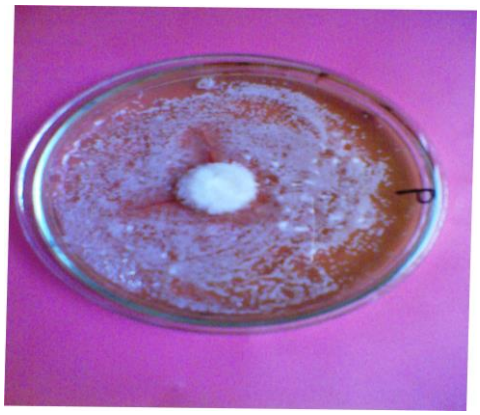

Fig.(11): the effect of polymer on the fungal *Candido albicans.*

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الملخص:

حضر البولي(فنيل كلورايد) الهلامي من تفاعل ٩٩% البولي(فنيل الكحول) المائي مع كلوتيرالدهايد كعامل تشابكي. حمل الهلام مع مضادات فطرية ودرس معدل اطلاق الادوية من الهلام من خلال استخدام تقنية الاشعة فوق البنفسجية.