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IN VITRO RELEASE OF CHLORAMPHENICOL SODIUM SUCCINATE FROM EUDRAGIT POLYMERIC FILMS

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تم اختبار أفلام بوليمرية من الايدراجيت رل كأنظمة للانطلاق البطئ لكلورامفينكول سكسينات الصوديوم والذى استخدم بتركيز ١٠٪ وزن/وزن. وتم تطرية هذه البوليمرات بالجلسيرول ثلاثى الاسيتات ، أو جليسرول ثلاثى البيتوريات أو البروبيلين جليكول أو ثنائى ميثيل الفيثاليات وتحضيرها. ووجد أن زيادة انطلاق العقار يمكن ترتيبه على النحو التالى: جليسرول ثلاثى اسيتات > بروبيلين جليكول > ثنائى ميثيل الفيثالات > جليسرول ثلاثى البيتوريات.

كما تم دراسة تأثير تركيز العقار الابتدائى وتركيز جليسرول ثلاثى الاسيتات على الانطلاق من فيلم البوليمر. ووجد أن ثابت معدل الانطلاق يتناسب مع تركيز العقار ومحتوى الفيلم من جليسرول ثلاثى الاسيتات. كما تم تحضير أفلام من نسب مختلفة من بوليمر ايدراجيت رل ، رس ووجد أن العقار ينطلق بسرعة أكبر باستخدام نسب ٨:٢ من رس:رل. وقد تم دراسة تأثير إضافة الهيدروكسى بروبيل ميثيل سليلوز على أفلام ايدراجيت رس على انطلاق العقار ووجد أن أحسن تركيز هو ١٠٪ وزن/وزن. ووجد أن انطلاق الدواء يتبع نموذج هيجوشى.

وتم دراسة أقل تركيز لتثبيط البكتيريا من الأصناف الاستافيلوكوكس أوريس ، باسيلس سيريس، إيشيرشيا كولاى والكانديدا البيكانس ووجد أن أقل تركيز لتثبيط البكتيريا يقع ما بين ٧ ، ١٢ ميكروجرام/مل. وأن قطر منطقة التثبيط للعقار من الفيلم رل/رس (٨:٢) أعلى منها من الشاش الطبى وأن النشاط ضد البكتيريا للكلورامفينكول سكسينات الصوديوم قد ازداد من فيلم رل:رس (٨:٢) والمطرى بـ ٢٠٪ جليسرول ثلاثى الاسيتات فى وجود ١٠٪ توين ٨٠.

Eudragit polymeric films were investigated as potential drug delivery systems for the controlled release of chloramphenicol sodium succinate (CSS). CSS at 10% w/w in polymeric film of Eudragit RL100, plasticized with glycerol triacetate (GTA), glycerol tributyrate (GTB), propylene glycol (PG) or dimethyl phthalate (DMP) was prepared. Increasing drug release rate from Eudragit RL100 films due to plasticizers could be arranged as follows: GTA > PG > DMP > GTB. The release of CSS in polymeric film of Eudragit RL100 was studied as a function of initial drug concentration and GTA content. The release rate constant of the drug was found to be proportional to drug concentration and GTA content in the film. CSS films composed of different ratios of Eudragit RS100 and Eudragit RL100 were prepared. The results show that CSS release from film containing Eudragit RS100/RL100 at a ratio of 2:8 was higher than those of the other polymer ratios. The effect of addition of different concentrations of hydroxypropyl methylcellulose (HPMC) to Eudragit RS100 films on the release rate of CSS was also studied. The best concentration of HPMC on increasing the release rate of the drug was found to be 10% w/w. The results indicate that drug release from matrix follows a diffusion-controlled model.

The minimum inhibitory concentration (MIC) of CSS for Staphylococcus aureus, Bacillus cereus, E. coli and Candida albicans was investigated. Results indicated that MIC of CSS was ranged from 7 to 12 μg/ml. The inhibition zone dimaeters of CSS film composed of Eudragit RS100/RL100 (2:8) were higher than that of CSS gauze dressing. In-vitro antimicrobial activity of CSS was enhanced in Eudragit RS100/RL100 film plasticized with 20% GTA in presence of 10% Tween 80.

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INTRODUCTION

Incorporation of drugs in inert polymer films during their manufacture affords a possible method of achieving controlled release. Such products can be adapted to topical, oral, and other routes of administration by utilizing them directly or in the form of coatings.[1-3] Drug release may be altered by variations of the dimensional parameters of the film, the polymer matrix material, and the drug concentration in the film.

Eudragits RL and RS are copolymers synthesized from acrylic and methacrylic acid esters which contain a low level of quaternary ammonium groups. The RL polymer contains a greater molar ratio of these ionizable groups, which causes it to be more readily permeable than the Eudragit RS.[1] Jenquin *et al.*[4,5] studied the release of salicylic acid and chlorpheniramine maleate from acrylic resin films. Topical polymeric films of acrylic resin have been reported by various investigators.[6-8]

Plasticizers are usually added to polymeric films to enhance their flexibility and to reduce their brittleness.[9,10] Plasticizers can also modify the physicochemical and mechanical characteristics of the film and influence the permeability rate of certain molecules.[11] Okor,[12] reported that the inclusion of glycerol tributyrate in film composition can enhance the permeability of the hydrophilic films.

Eudragit RS alone forms a very britlle film. Addition of hydrophilic polymers such as HPMC and polyethylene glycol not only alters drug release pattern from film, but also yields a flexible film.[13,14]

Chloramphenicol sodium salt is available in various topical formulations.[15] It is used for the treatment of several skin diseases. It has a short duration of action and frequent applications are usually required. On this basis, it is of interest to formulate CSS in polymeric films, which may control the drug delivery over a reasonable period of time.

The purpose of this study was to investigate the release of CSS from polymeric films composed of Eudragit RL100, Eudragit RS100 or different ratios of Eudragit RS100/RL100. The effect of different plasticizer concentrations and drug loading in the Eudragit RL100 films on the release rate of CSS was studied. Also, the effect of HPMC incorporation in Eudragit RS100 films on drug release was investigated. *In-vitro* evaluation of antimicrobial activity of CSS from the Eudragit polymeric films with different additives (plasticizer and/or enhancer) was also studied.

EXPERIMENTAL

Materials

Chloramphenicol sodium succinate (kindly provided by CID Company for Pharmaceutical Industries, Egypt), Eudragit RS100 and RL100 (Rohm Pharma, GMBH Darmstadt, Germany), Glycerol triacetate (GTA), Glycerol tributyrate (GTB), Dimethyl phthalate (DMP) and Tween 80 (Merck, Suhuchardt, Munchen, Germany), Hydroxypropyl methylcellulose HPMC (Sigma Chemical Co., St. Louis, MO.), Citric acid, Propylene glycol PG, Sodium citrate and Acetone (El-Nasr Company, Adwic, Egypt). Nutrient agar and Nutrient broth were obtained from (Sigma Chemical Co., St. Louis, Mo., USA). The micro-organisms used in this study were: *Staphylococcus aureus* (ATCC 25923), *E. coli* (ATCC 25928), *Bacillus cereus* (ATCC 7978), and *Candida albicans* (ATCC 753).

Equipment

USP dissolution test apparatus II (Paddle), SRI 6-flask (Hanson research, USA), Spectrophotometer, UV-1601 (Shimadzu Co., Japan) and pH meter (Tesamaster, Tesa, Switzerland).

Film preparation

Polymer films were prepared by solvent casting with acetone into a Teflon disc (area= 28.26 cm^2) placed on a flat surface at room temperature. Chloramphenicol sodium succinate (10% w/w) and polymer (Eudragit RL100, Eudragit RS100 or different ratios of Eudragit RS100/RL100) were accurately weighed and then dispersed in acetone. The mixture was

stirred until the polymer was dissolved and the solution well mixed. Film solution was distributed and acetone was allowed to evaporate at room temperature. Complete evaporation was obtained by drying for 24 hrs to a constant weight. The films were stored in a desiccator (anhydrous calcium chloride) for 24 hrs and then placed in a sealed container until time of use. Just prior to the drug release studies, the outer rim of the aluminum boat was removed leaving only a 5-mm tip. To avoid differences in drug release experienced with films of inconsistent curing due to unevaporated solvent or film aging, the drug release properties of all films were investigated 7 days after preparation. Eudragit RL100 films were prepared containing different concentrations of CSS, and the release profile for each film was examined. Eudragit RL100 films containing different types of plasticizers (20% w/w), namely GTA, GTB, DMP and PG were prepared. Eudragit RS100 films containing different concentrations of HPMC were investigated.

The thickness of the dried films was measured at five different places using a micrometer (Mitotoyo, Japan) and the mean values were calculated (230 mm$\pm$2). The uniformity of drug content of the films was determined, based on the drug weight ratios of drug and the used polymers, by a spectrophotometric method.

Drug release studies

The dry films of known thickness were cut to rectangular shape (10 cm^2) using a glass template and fixed over a glass plate with silicone adhesive. The plate was immersed in a 200 ml of citrate buffer solution (pH= 5.0) maintained at a temperature of 37°$\pm$1. Then the paddle was positioned at a distance of 2.5 cm from the surface of the glass plate and regulated to rotate at a speed of 60 rpm. Aliquots of the samples (5 ml) with withdrawn at predetermined time intervals and assayed spectrophotometrically at λ_{max}= 278 nm for CSS. After each sampling, an equal volume of buffer solution (pH= 5.0) preheated at 37° was added to the dissolution medium to maintain a constant volume. The experiment was done in triplicate and mean value was calculated. The release rate constants were calculated from the linear plots of cummulative amount of drug released versus square root of time.

Culture and *in-vitro* susceptibility test

Films formed of Eudragit RS100 : Eudragit RL100 in a ratio of (2:8) and plasticized with 20% of GTA in the absence and presence of 10% Tween 80 were prepared. Tube dilution technique was used for measuring the minimum inhibitory concentration (MIC)[16] and the diffusion method was used for determining the sizes of inhibition zones.[17] The four tested microorganisms, namely *Staphylococcus aureus, E. coli, Bacillus cereus* and *Candida albicans* were grown at 37° for 24 hrs on nutrient agar plates. The four previously mentioned microorganisms were inoculated onto the medium to give approximately 10^6 cells/ml for each microorganism. The prepared suspensions were diluted with sterile saline solution and adjusted to pH 6.5. Media growth were recorded after 24 hrs incubation at 37°. Susceptibility tests were done against each individual organism in an aseptic conditions. The minimum inhibitory concentration was considered as the lowest concentration of the drug in μg/ml that prevents *in-vitro* growth. Control was also done in parallel with the test for each sample. The mean of three readings was determined.

The prepared chloramphenicol-Eduragit films containing different drug concentrations (1, 2.5, 5 and 10%) and plasticized with GTA (20%) or containing Tween 80 (10%) were tested for antimicrobial activity against the previously mentioned microorganisms. The tested drug concentrations were completely dissolved in the polymer and this was obvious from the apparent transparency of these films. Also, chloramphenicol gauze dressings were prepared and subjected to the *in-vitro* susceptibility test.

For inoculation of nutrient broth, a volume of 0.1 ml bacterial culture was placed onto the surface of nutrient agar plate. Discs of chloramphenicol-Eudragit films and gauze dressing were placed and gently pressed down on the surface of agar plate. Plates were incubated for 24-48 hr at 37° and zones of inhibition were measured.

RESULTS AND DISCUSSION

Effect of drug concentration

The solvent casting method used to prepare Eudragit RL100 films containing CSS produced clear, colorless stabs. There were no visible signs of drug crystallization in up to 10% w/w CSS-containing films. Drug release and recovery results showed that solvent casting produced films having very uniform drug concentrations.

The percent loaded drug release versus time for Eudragit RL100 films containing varying concentrations of CSS is shown in Fig. (1). *In-vitro* release followed Higuchi-controlled model as its correlation coefficients (r= 0.996-0.954) predominates over zero and first-order kinetics, Table 1. Figure (2) describes the drug release from monolithic dispersions as being linear proportionality between the amount of drug released and the square root of time over almost the entire release curve.[18] These linear plots appear to indicate that the drug release from these films is diffusion drug controlled. The release rate constant of the drug is proportional to the drug concentration. This could be explained by assuming that matrix porosity necessary for the diffusion pathways may be due to the pores created by the dispersed drug. Therefore, increasing drug concentration in the film would result in increasing the degree of internal porosity. This would consequently increase the film area exposed to the release medium.[19,20]

Effect of polymer ratio

Eudragit RS100 and Eudragit RL100 are anionic polymers of methacrylic acid and methyl methacrylate. Eudragit RS100 is less permeable than Eudragit RL100 due to its lower content in quaternary ammonium groups (RS100 1/40 ammonium/ester; RL100 1/20 ammonium/ester). Because of the unlimited miscibility of the two types, the permeability of the films can be adjusted for the diffusion of drugs on the basis of their solubility and the desired rate of release. The release rate constant (K) of CSS (10% w/w) from Eudragit RL100 film was 4.3 times higher than that with Eudragit RS100 film. Also, the half-lives of Eudragit RS100 was 29 times longer than that with Eudragit RL100. Although Eudragit RL100 is highly hydrated polymer, no visual tearing or rupturing of the films was observed until all of the drug was released.

Figure (3) shows the effect of film composition on the release rate of CSS from films containing a 10% w/w initial drug concentration. It is clear from this figure that as the Eudragit RL100 content of the film increased, the release rate of the drug also increased. Accordingly, an increase of the release rate constants could be observed upon increasing the fraction of Eudragit RL100 (Table 2). This may be attributed to the high degree of hydration of the hydrophilic polymer leading to increase the porosity and the formation of hydrated channels.[8] Also, linearity with high correlation coefficient resulted on plotting the percentage of drug release (Q) against the square root of time is in agreement with Higuchi-diffusion model.

Figure (4) shows the existance of linearity with high correlation coefficient between the logarithmic value of release rate constant (log K) and the fraction of the more hydrophilic polymer greatly confirms that the release of CSS from Eudragit films follows Higuchi-diffusion model. Similar result were obtained by Borodkin *et al.*[21] who stated that there was a linear relationship between log K and fraction of HPMC in films composed of polyvinyl acetate / HPMC containing either methapyrilene or salicylic acid or phenobarbital.

Effect of plasticizer

Two proposed factors could be taken into consideration to explain the effect of plasticizer content on drug release profile. The first is that the plasticizer may decrease the degree of compactness of the polymeric matrix due to bonding to the polymer molecules and forming pores through which the drug leaches out. The second factor is the solubility of the plasticizer in water through hydrogen bonding leading to an increase in the hydrophilic properties of the matrices and formation of hydrated channels.

Table 1: Influence of film composition, initial drug concentration and type of plasticizer on the release rate constant of chloramphenicol sodium succinate from Eudragit RL100 films.

Film composition	Intial drug conc. (% w/w)	Different* type of plasticizer (20% w/w)	GTA* (% w/w)	Release rate constant ($mg/cm^2.min.^{½}$)	Correlation coefficient (r)	Half-life t½ (min.)
Eudragit R100	1			1.038	0.954	2266.1
	2.5			1.292	0.974	1418.4
	5			2.631	0.991	364.7
	10			3.620	0.996	124.4
Eudragit RL100		GTA		3.423	0.990	197.4
		PG		1.805	0.971	525.2
		DMP		2.113	0.997	515.8
		GTB		1.478	0.991	866.1
Eudragit RL100			0	3.620	0.996	124.4
			5	2.196	0.971	517.7
			10	2.689	0.990	288.2
			15	2.834	0.981	185.3
			20	3.423	0.990	197.4

*Chloramphenicol sodium succinate= 10% w/w.

Table 2: Influence of film composition and the addition of hydroxypropyl methylcellulsoe (HPMC) on the release rate constant of chloramphenicol sodium succinate, CSS (10% w/w).

Film composition	HPMC conc. (% w/w)	Release rate constant ($mg/cm^2.min.^{½}$)	Correlation coefficient (r)	Half-life t½ (min.)
Eudragit RS100/RL100 ratio				
10:00		0.656	0.995	5265.7
8:2		1.150	0.960	1816.0
6:4		1.427	0.968	118.9
4:6		1.934	0.993	556.2
2:8		2.875	0.991	310.9
00:10		2.965	0.960	181.7
Eudragit RS100				
	0	0.656	0.995	5265.7
	5	2.057	0.963	737.9
	10	3.220	0.959	241.9
	20	2.627	0.970	67.5

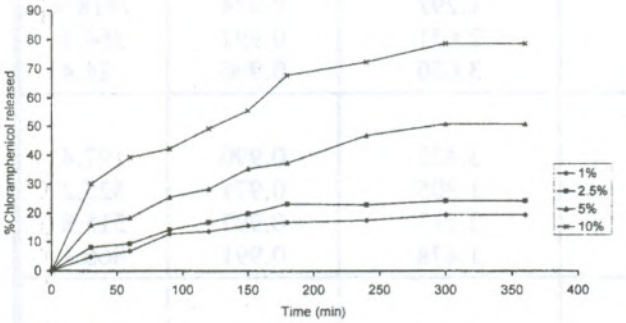

Fig. 1: Effect of chloramphenicol sodium succinate concentrations in Eudragit RL100 film, on the drug release.

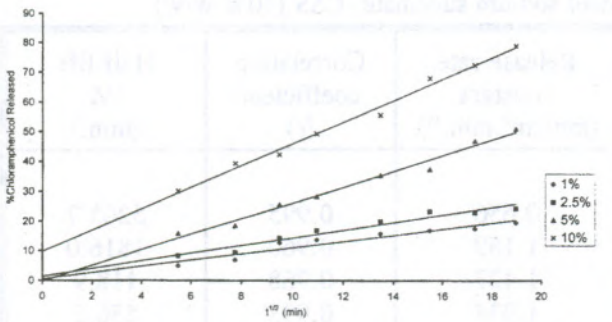

Fig. 2: Higuchi-diffusion release profile for films composed of Eudragit RL100 and containing different concentrations of chloramphenicol sodium succinate (% w/w of dry film).

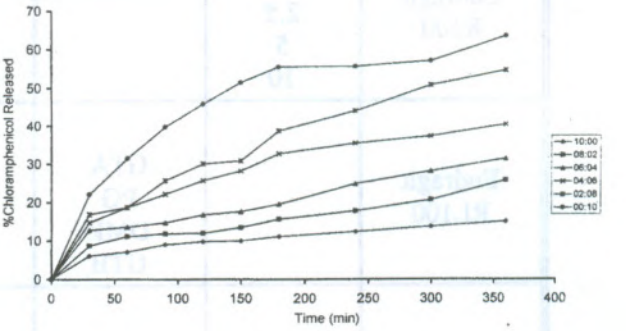

Fig. 3: Drug release from films containing various ratios of Eudragit RS100 / Eudragit RL100 loaded with (10% w/w) chloramphenicol sodium succinate.

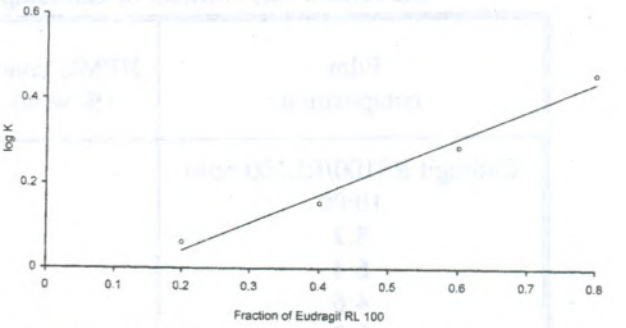

Fig. 4: Relationship of log K (release rate constant) to the fraction of Eudragit RL100 for films containing (10% w/w) of chloramphenicol sodium succinate at different ratios of Eudragit RS100 : RL100.

This could explain the higher effect of the water soluble plasticizers (GTA and PG) on the drug release rate from Eudragit RL100 polymeric matrix. While, DMP and GTB were used as water-insoluble plasticizer.

Figure (5) shows the effect of different types of plasticizers on the release profile of CSS from Eudragit RL100 polymeric films. The effect of plasticizers could be arranged as follows: GTA > PG > DMP > GTB. It could be also noticed that the increase in GTA plasticizer concentration would result in increasing the release rate constant of the drug (Fig. 6 and Table 1).

Upon diffusion of the buffer inside the film, CSS would diffuse through the hydrated voids created by those water-insoluble plasticizers. This may explain the smaller amount of drug released from Eudragit RL100 films plasticized with either DMP or GTB. Similar results were obtained by Ismail *et al.*[6] and Salama *et al.*,[8] who studied the release of salicylic acid and chlorphenesin from Eudragit films containing different types of plasticizers.

Effect of addition of hydroxypropyl methylcellulose (HPMC)

Formulation of polymeric films may require the addition of hydrophilic polymer (HPMC) to replace a portion of the Eudragit RS100 polymeric film to modify drug release rate. Figure (7) shows the effect of addition of HPMC (different concentrations) to Eudragit RS100 films on the release rate of CSS. The increase in drug release with increasing proportions of HPMC in the matrix could be explained on the basis of leaching of the hydrophilic polymer.[21] No visual dissolution of the films was observed until all of the drug was released. The highest correlation coefficients were obtained with Higuchi-equation (Table 2), indicating diffusion-controlled drug release. The dissolution profile, plotted as a square root of time basis was acceptably linear for up to 80% drug release at different concentrations of HPMC. These results confirm the findings of Ford *et al.*[22] that there is a linear relationship between drug release and square root of time for HPMC matrix containing promethazine hydrochloride. Also, the replacement of portions of HPMC within the matrices by insoluble or soluble diluents increased the release rates of the drug.

***In-vitro* antimicrobial activity of CSS polymeric films**

The results of the *in-vitro* antimicrobial activity of CSS against microorganisms were shown in Table (3). The minimum inhibitory concentration (MIC) of CSS ranged from 7-12 μg/ml. It has been concluded that these microorganisms were susceptible to CSS.[15]

A quantitative comparative study of the antimicrobial activity of CSS / Eudragit polymeric film and gauze dressing, each containing 1% CSS, was performed and the results were shown in Table (4). It was found that CSS film showed a higher response in the inhibition zone than gauze dressing.

The sizes of inhibition zones for CSS/Eudragit polymeric films at 1, 2.5, 5 and 10% concentration showed a dramatic increase in the inhibition zone sizes with increasing CSS concentrations as shown in Table (5) and Fig. 8. The incorporation of 10% Tween 80 (as enhancer) into Eudragit RL100/RS100 (8:2) plasticized with 20% w/w GTA, resulted in higher response in the inhibition zone sizes for the drug (Fig. 9). The inhibition zone sizes reflected quantitive concentration gradient established by diffusion of the drug through a given medium and the succeptibility of the tested organisms. These polymeric films exhibited rapid drug delivery due to presence of hydrophilic components in the film such as Eudragit RL100, GTA and Tween 80. All these components promote CSS diffusion through the medium and thereby enhance its antimicrobial activity.

In conclusion, the use of polymeric films as drug delivery systems enhanced CSS *in-vitro* antimicrobial activity and it was recommended to use CSS polymeric films for topical treatments of cuts and wounds.

Table 3: Minimum inhibitory concentration (MIC) of chloramphenicol sodium succinate powder (tube dilution method).

Microorganism	MIC (μg/ml)
Staphylococcus aureus	12
Bacillus cereus	12
E. coli	10
Candida albicans	7

Table 4: Comparison of inhibition zones of chloramphenicol sodium succinate (10% w/w) film composed of Eudragit RS100/RL100 (2:8) with CSS guaze dressing.

Microorganisms	Zones of inhibition (diameter in mm)		
	Gauze 1% dressing	Film 1%	Plain film
Staphylococcus aureus	5	7	0
Bacillus cereus	7	10	0
E. coli	6	13	0
Candida albicans	8	9	0

Table 5: Antimicrobial effect of chloramphenicol sodium succinate (10% w/w) polymeric film composed of Eudragit RS100/RL100 (2:8) plasticized with 20% GTA.

Drug concentration (% w/w)	Zones of inhibition (diameter in mm)			
	Staphylococcus aureus	*Bacillus cereus*	*E. coli*	*Candida albicans*
Without Tween 80				
1	2	8	6	1
2.5	4	15	17	12
5	5	14	18	18
10	15	19	20	19
With Tween 80 (10%)				
1	4	10	13	9
2.5	7	17	15	13
5	10	17	17	21
10	17	22	18	24

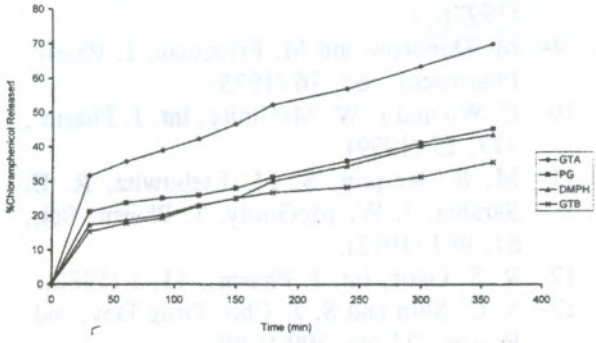

Fig. 5: Effect of different types of plasticizers (20% w/w) on the release of chloramphenicol sodium succinate (10% w/w) from Eudragit RL100 films.

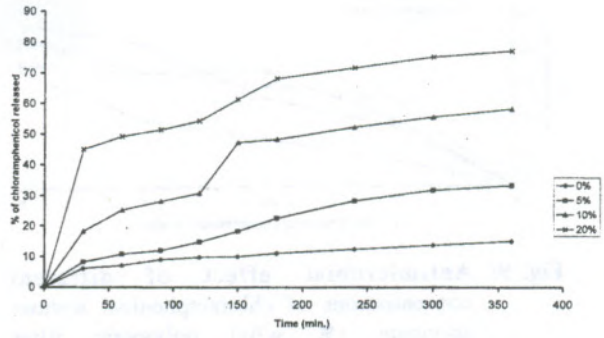

Fig. 7: Effect of different concentrations of HPMC (% w/w) on the chloramphenicol sodium succinate (10% w/w) from Eudragit RS100 films.

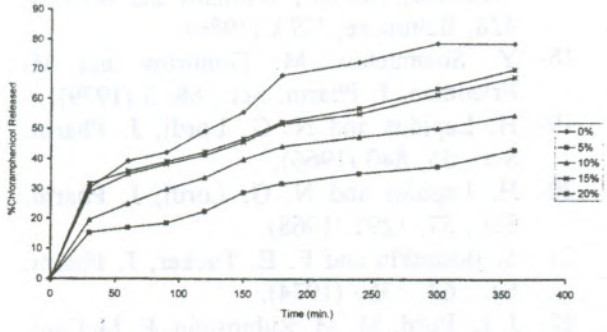

Fig. 6: Effect of different concentrations of glycerol triacetate on the release of chloramphenicol sodium succinate (10% w/w) from Eudragit RL100 films.

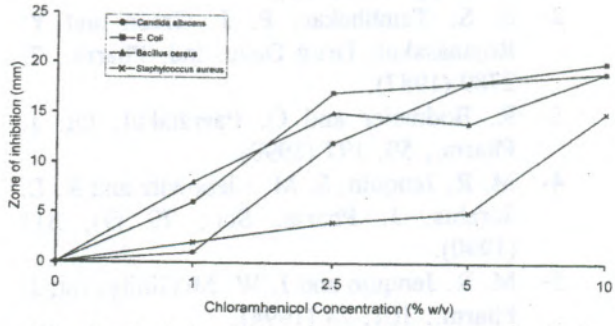

Fig. 8: Antimicrobial effect of chloramphenicol sodium succinate polymeric films composed of Eudragit RL100 and RS100 (8:2) plasticized with 20% GTA.

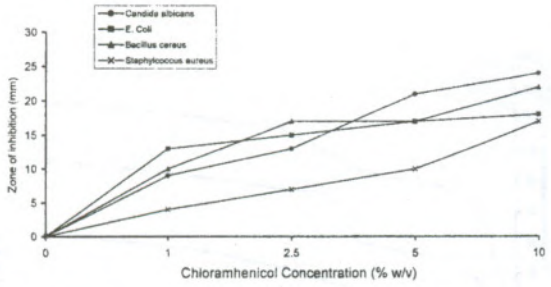

Fig. 9: Antimicrobial effect of different concentrations of chloramphenicol sodium succinate (% w/w) polymeric films composed of Eudragit RL100 and RS100 (8:2) plasticized with 20% GTA in the presence of 10% Tween 80.

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