Bull. Pharm. Sci., Assiut University, Vol. 28, Part 2, December 2005, pp. 205-211.

PHONOPHORESIS OF THEOPHYLLINE THROUGH CELLOPHANE MEMBRANE AND RABBIT SKIN

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تتناول الدراسة تأثير الموجات فوق الصوتية على نفاذية الثيوفيلين خلال غشـــاء السيلوفان وجلد الأرنب و ذلك باستخدام الطريقة المستمرة للموجات وبقوة (٠,٥ ، ١ ، ١,٥و٢) وات/ سم^{٢} بتردد ٨٠٠ك.هرتز لمدة ساعة. كما تم تقيم القواعد الهلامية المختلفة وهى هيدروكسى بروبيل ميثيل سيليلوز ، كربوكسى ميثيل سيليلوز الصوديوم و الكربابول ٩٣٤ بتركيزات مختلفة من ١ ٤% . و قد أظهرت نتائج الدراسة أن تأثير الموجات فوق الصوتية على نفاذية الثيوفيلين خلال جلد الأرنب بمعدل أقل منه في غشاء السيلوفان. كما أوضحت النتائج الزيادة الطردية في نفاذية العقار بزيادة قوة الموجات فوق الصوتية . كما وجد أن زيادة تركيزات المعقدات فى القواعد الهلامية يؤدى إلي انخفاض نفاذية الدواء وبناءا على دراسات النفاذية واللزوجة تم اختيار أفضل المعقدات بأنسب التركيزات وتم حساب قيم التدفق للعقار من هذه المعقدات وهي (٥,٩٩ ، ٣,٦٩ ، ٢,٤ميكروجرام/ دقيقة.سم^{٢}) بالنسبة إلي ٢% هيدروكسى بروبيل ميثيل سيليلوز، ٤% كربوكسى ميثيل سيليلوز الصوديوم و ٢% كربابول ٩٣٤ على التوالي. وقد تم التوصل إلي أن انطلاق العقار من القواعد الهلامية لهيدروكسى بروبيل ميثيل سيليلوز يتبع معادلة الرتبة الصفرية بينما انطلاقة من كربوكسى ميثيل سيليلوز الصوديوم والكربابول طبقا لمعادلة الرتبة الأولى ونظام انتشار هيجوشى على التوالى.

The influence of ultrasound waves upon the permeation of theophylline through cellophane membrane and rabbit skin was studied in vitro. Sonication was carried out with continuous mode at intensities (0.5, 1, 1.5, & 2 W/cm^2) at constant frequency 800 KHz for one hour. Different gel formulations with (hydroxypropylmethyl cellulose, sodium carboxymethylcellulose and carbopol 934P) in different concentrations (1-4% w/w) were utilized. Phonophoresis of theophylline through rabbit skin were significantly less than that obtained with the cellophane membrane. Ultrasound application has showed a significant increase in the amount of theophylline permeation with increasing intensity. For all the tested gelling agents, the amount of drug released was decreased by increasing polymer concentrations. The Flux values were 5.99, 3.69 & 2.4 (µg/min cm^2) for 2% HPMC, 4% Na CMC and 2% carbopol 934P gels respectively. It was found that drug release from HPMC gels obeys Zero-Order model while its release from Na CMC & carbopol 934P were fitted with First-Order and Higuchi-diffusion model respectively.

INTRODUCTION

Enhancing the delivery rate of drugs across the skin to attain significant therapeutic level is a great challenge due to low permeability of the skin to many drugs. Several approaches have been applied to enhance percutaneous drug absorption through the skin such as chemical enhancers,[1,2] iontophoresis,[3,4] electroporation[5,6] and phonophoresis.

Phonophoresis was first reported nearly 40 years ago. It has been defined as the migration of drugs through the skin under the influence of ultrasound waves.[7] Many authors have reported the positive effect of ultrasound application upon the delivery of many drugs; heparin,[8] Plasmid DNA,[9] Clarithromycin,[10] using different devices and different ultrasound conditions. However, other workers[11,12] found no significant flux enhancement. It was evident that the effect of phonophoresis depends on the nature of the drug, the type of the base and the conditions of ultrasound applications.[10]

Although theophylline is used topically to relieve apnea in neonate, But the therapeutic drug concentrations being achieved for up to 7 days after topical applications.[13,14] Enhancing delivery of theophylline is, therefore, desirable

Received in 24/3/2005 & Accepted in 6/6/2005

to obtain rapid action, zero-order kinetics, minimum side effects and better compliance.

The aim of this study is to justify the ultrasound effects on the delivery of theophylline from different gel bases through cellophane membrane and rabbit skin to determine the possibility of developing good transdermal systems.

EXPERIMENTAL

Materials

Theophylline (Batch No 142634) (Memphis pharmaceutical Co., Cairo, Egypt), hydroxypropylmethyl cellulose (HPMC) (Methocel K 100 M) (Dow Chem. Co., Midland MI), sodium carboxymethyl cellulose (Na CMC), and triethanolamine (El Naser Chem. Co., Egypt). Carbopol 934P (USP 400 cps BDH, Ltd, England). Cellophane membrane, Spectrapor, MW. Cutt-off 1200-1400 (Fisher Sci. Co., USA), All other chemicals were of analytical grade.

Apparatus

UV/VIS spectrophotometer (JASCO, V-530, Japan). Electric balance (Denver instruments Co., USA), pH-meter (Pye Unicam LTD, model 292, Cambridge, England. Centrifuge (DT. 51 Germany). Ultrasonic Therapy Apparatus (CSL-1, Shanghai, China), Rotary Viscometer (Roto Visco, Germany). Modified diffusion cell (Fig.1).

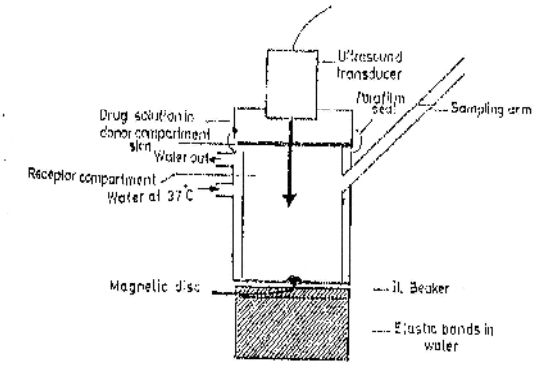

Fig. 1: Schematic diagram of the diffusion cell and transducer.

Methodology

Rabbit skin

Rabbit skin samples were obtained from abdominal skin of female rabbits (2-2.5 kg body weight). The skin was excised just prior to experiments. The hair removed and the skin cleaned with saline solution (0.9% w/w) to remove all visceral debris.

Preparation of gel formulations

The ingredients of all tested gel formulations containing 1% of theophylline are shown in Table (1). For preparation of each batch, the predetermined weight of each gelling agent (HPMC, Na CMC, or Carbopol 934P was sprinkled gently, with continuous stirring on the surface of distilled water, previously boiled and cooled. The dispersions were set-aside over night to form a gel. The drug was dissolved in

Table 1: Composition of different gel formulations containing 1% w/w theophylline.

Ingredients	Percent concentrations (w/w)								
	Gel I	Gel II	Gel III	Gel IV	Gel V	Gel VI	Gel VII	Gel VIII	Gel IX
Theophylline	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
HPMC	2	3	4	-	-	-	-	-	-
Na CMC	-	-	-	2	3	4	-	-	-
Carpobol 934	-	-	-	-	-	-	1	2	3
Triethanolamine	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Distilled Water to	100	100	100	100	100	100	100	100	100

another small portion of distilled water containing triethanolamine (2.5 ml) prior to incorporation into the tested formulation. The total weight is adjusted to 100 gram with distilled water.

Rheological measurement of tested formulations

For all gel formulations the rheological measurements were carried out. The entrapped air was removed from tested formulations by centrifugation of the prepared gel at 20,000 rpm for 10 min at 20° and stored at room temperature for at least 24 h before testing. Rheological measurement of the different formulations were adapted with shear rate controlled viscometer at 37°± 0.2°. The sensor system consists of a cup and bell-shaped rotor. The viscosity determined by the equation

$$\eta = \frac{G.S}{n} mPa.s$$

Where η is the viscosity, G instrument factor (10^3 A/M m Pa. s/ scale grade. min), S is the torque value and n is present test speed.

Permeability studies

The permeability studies were carried out according to the method adapted previously[10] using a specially designed diffusion cell, which allow the introduction of ultrasound probe into the donor compartment (Fig.1).

The permeability experiment was performed either with cellophane membrane or rabbit skin barrier. The cellophane membrane was wetted by soaking in distilled water, dried with filter paper and mounted in the mouth of donor compartment. While the rabbit skin barrier was mounted on the mouth of the donor half- cell between the cell flange and faceplate. The barrier was allowed to equilibrate for 30 minutes in the recipient vehicle.

The length of the donor half cell was 10 cm with an inside diameter of 6 cm and the volume of the donor cell was 246.4 ml. The total barrier surface available for diffusion was 28.31 cm^2. The donor cell was positioned in the middle of 500 ml-beaker serving as receptor compartment by a stirring with a magnetic bar 5-cm from the barrier surface at a speed of 90 rpm. The permeability experiments are conducted with 400 ml of phosphate buffer 7.4 in the receiver chamber and 10 gram of 1% theophylline gel in the donor chamber. The cell was maintained at 37°± 0.2°.

The ultrasound probe equipped at a flat tip, having 7.071 cm^2 of the surface was centrally positioned and submerged into the gel in the donor compartment. Ultrasound is applied in the following conditions; Different intensities (0.5, 1, 1.5, and 2 w/cm^2), continuous mode, 800 KHz frequency, stationary application, for one hour duration. Samples are withdrawn from receiver compartment at predetermined time intervals and replaced with fresh buffer pH 7.4. Theophylline concentration is determined spectrophotometrically at predetermined λ max (271 nm).

The total permeability coefficient (*P*) is calculated at steady state under sink condition. Experiment is run long enough so that the steady-state portions are typically around 3 to 5 times longer than the lag times. The method reported by Yoneto *et al*.,[15] was used to determine the free diffusion coefficients and analysis of permeation data. The permeability coefficient (*P*) was then calculated according to the equation:

$$P = \frac{1}{AC_D}\frac{dQ}{dt}$$

Where *A* is the diffusion area of the glass disk, C_D is the donor concentration, and (*dQ/dt*) is the slope of the linear region of the plot of the amount of theophylline in the receiver chamber versus time. Steady-state Fluxes (*J*) were calculated from the slopes of the best–fit regression line between the treatments.

Statistical and kinetic treatment of the data

The obtained results were statistically analyzed following the analysis of variance and t-test (at 0.05 significance level) by SPSS computer program (SPSS 0.8 for windows).

All the *in-vitro* release data were analyzed according to zero-order, first order[16] and Higuchi model[17] to determine the mechanism of drug release. The best linear relation was based on the correlation coefficient (r) for the parameter studied.

RESULTS AND DISCUSSION

An *in-vitro* experiment, by modified diffusion cell Figure (1) is an important

controlled environment to quantify the parameters of phonophoresis, gel type and concentrations suitable for theophylline formulations.

Figure (2) illustrates the permeation of theophylline through cellophane membrane and rabbit skin without ultrasound and with different ultrasound intensities (0.5, 1, 1.5 &2 W/cm^2) in 2% HPMC gel. It was found that; theophylline was transported from both membranes in a measurable quantity after one hour without sonication. Ultrasound application has showed a proportional increase in the amount of theophylline passing through both membranes. The increased drug diffusion by increasing ultrasound intensities may be explained as ultrasound produces both thermal and non thermal effects. The non thermal effects are cavitations, radiation pressure and acoustic microstreaming.[9] These mechanisms may influence the vehicle diffusion coefficient or membrane itself and depended on the duration and intensity of sonication.[7]

The higher permeation of theophylline through cellophane membrane than rabbit skin may be due to the different nature of both barriers; cellophane membrane is easily hydrated by dissolution medium causing the pores of the membrane are much more wider. However, the rabbit skin is a complex thick structure with low permeability. These finding were found to be in agreement with those obtained by Muller & Kreuter, 1999[18] on the transport of captopril through artificial membrane and hairless mice skin.

Viscosity measurements of the prepared gel formulations with varying polymer concentrations at 37°± 0.2° was shown in Table (2).

Table 2: Mean* viscosity measurements of different gel formulations at 37° ± 0.2° [Mean ±SD].

Gel formulations concentration (% w/w)	Mean viscosity (m.Pa)
HPMC 2%	12061 ±321
HPMC 3%	20732 ± 223
HPMC 4%	23358 ± 521
Sod.CMC 2%	01980 ± 221
Sod.CMC 3%	02061 ± 113
Sod.CMC 4%	10740 ± 331
Carbopol 1%	12941 ± 220
Carbopol 2%	30416 ± 411
Carbopol 3%	31220 ± 110

* Mean of three determinations

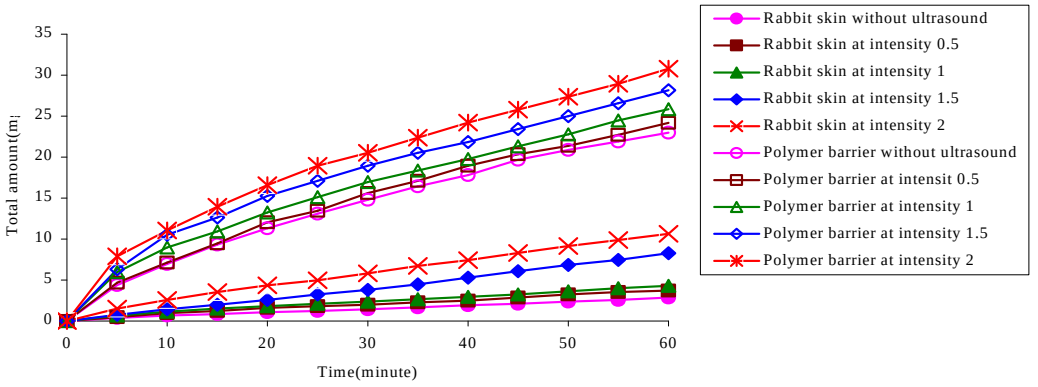

Fig. 2: The permeation of Theophylline through polymer barrier and rabbit skin using 2%HPMC gel at different intensities of ultrasound.

Figure (2) : The permeation of Theophylline through polymer barrier and rabbit skin using 2%HPMC gel at different intensities of ultrasound

It was revealed that the carbopol 934P gels had a viscosity 12941, 30416 & 31220 m. Pa for 1, 2 & 3% concentrations respectively. These values were greater than that obtained from other two HPMC and Na CMC gels.

The observed higher increase in viscosity with increasing polymer concentrations is expected and can be explained by macro-molecular entanglement phenomena. Since higher concentrations increase the entanglement density (the number of intermolecular contacts per unit volume), the viscoelastic properties increase correspondingly.[19]

For carbopol 934P gel, the viscosity was 30416 and 31220 m Pa. for 2 and 3% concentration respectively. The concentration dependence of viscosity diminished above 2% concentration in which the elasticity of the gel did not gain on an increased concentration. Only the rheological measurements of Na CMC gel are affected on the addition of theophylline. As the gel is polar,with higher solubility, so the contribution of the drug affects the total ionic strength of gel system.[19] On the other hand, the viscosity of HPMC and Carbopol 934P gels is not affected by drug incorporation.

Table (3) illustrates the mean permeability coefficient of theophylline permeation through rabbit skin from gel formulations using different intensities of ultrasound. It was found that the permeability coefficients of theopylline (*P*) form tested gel formulations were increase with increasing ultrasound intensity. Comparison between different gels at ultrasound intensity 2 W/cm^2 was evaluated. The permeability of the drug from HPMC gel, were 0.59, 0.56 & 0.5 (g / cm^2. min x10^{-3}), and from Na CMC gel were 0.55, 0.45 & 0.36 (g / cm^2. min x10^{-3}) for 2, 3 & 4% w/w concentration respectively.

It was clear that a decrease in the permeation of the drug was parallel with increasing polymer concentration (Table 2).

Comparing the permeability of theophylline from different polymers at the same concentration illustrate that the permeability of the drug at 2% concentration were 0.59, 0.55 and 0.24 (g/cm^2.min x10^{-3}) for HPMC, Na CMC and Carbopol 934P respectively at intensity 2 W/cm^2. The highest values for the permeability from cellulose polymers may be attributed to the thixotropic properties of these gels. Both form a network like structures.

These networks were achieved by attraction between its molecules by hydrogen bonds or Van der Waal's forces.[20] The thermal effect of ultrasound may break these weak bonds causing liquefaction of gels with rapid drug release.

Table 3: Mean permeability coefficient of Theophylline through rabbit skin from gel formulations using different intensities of ultrasound.

*Mean permeability coefficient (*P*) (g / cm^2. min x10^{-3})					
Gel formulations (%w/w)	Ultrasound intensity (W/cm^2)				
	Control**	0.5	1	1.5	2
HPMC 2%	0.15±0.012	0.20±0.021	0.23±0.028	0.47±0.017	0.59±0.023
HPMC 3%	0.12±0.011	0.18±0.019	0.21±0.026	0.40±0.030	0.56±0.017
HPMC 4%	0.09±0.003	0.15±0.014	0.18±0.018	0.33±0.029	0.50±0.012
Sod.CMC 2%	0.12±0.013	0.18±0.019	0.21±0.022	0.41±0.032	0.55±0.025
Sod.CMC 3%	0.09±0.004	0.14±0.017	0.18±0.021	0.32±0.028	0.45±0.031
Sod.CMC 4%	0.07±0.002	0.10±0.013	0.16±0.017	0.24±0.025	0.36±0.034
Carbopol 1%	0.08±0.011	0.10±0.016	0.15±0.024	0.24±0.026	0.25±0.029
Carbopol 2%	0.07±0.004	0.09±0.008	0.10±0.012	0.18±0.023	0.24±0.031
Carbopol 3%	0.06±0.008	0.07±0.005	0.09±0.007	0.10±0.016	0.10±0.019

*Average of three determinations **without ultrasound applications

On the other hand, the lower permeability of theophylline from 2% Carbopol 934P gel may be due to good thermal stability of the gel, so that the gel viscosity is unaffected by temperature.[21] Also, Carbopol 934P gel posses higher viscosity (30416 m Pa.) due to increase the number and size of aggregates within the gel which may hinder drug permeation.

The gels which illustrate the higher permeability of theophylline through rabbit skin with an appropriate viscosity were 2% HPMC, 4% Na CMC and 2% Carbopol 934P gels. Figure (3) illustrates the mean Flux values for the selected gels compared to control. These values were 5.99, 3.69 & 2.4 (µg/min $cm^{2)}$ for 2% HPMC, 4% Na CMC and 2% Carbopol 934P gels respectively.

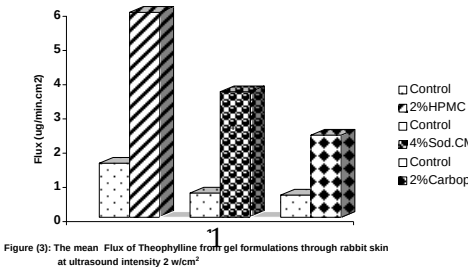

Figure (3): The mean Flux of Theophylline from gel formulations through rabbit skin at ultrasound intensity 2 w/cm²

Fig. 3: The mean Flux of Theophylline from gel formulations through rabbit skin at ultrasound intensity 2 W/cm^2.

Table (4) illustrates the kinetic evaluation of the theophylline release data under the influence of ultrasound intensity 2 W/cm^2 according to Zero-order, First-order and Higuchi diffusion model.

It is clear that the best linear relations and highest correlation coefficient is obtained with Zero-order model for HPMC gels at all polymer concentrations (r is not less than 0.9985).

The release of the drug in a manner independent on the concentration is highly desirable in the delivery of the drug from transdermal gel system. However, the release of theophylline from Na CMC gel obeys First-order kinetics (r is not less than 0.9984).

The best fitting linear relation with Carbopol 934P gels is observed with Higuchi-diffusion model (r is not less than 0.9987). This is may be due to the nature of carbopol 934P gel. The cross-linking of the gel by triethanolamine allows a greater number of pores from which the soluble drug can diffuse into the medium. These finding was agreed with those obtained from the release of niosomes from carbopol 934P gel which obeys Higuchi- diffusion model.[21]

Table 4: Kinetic modeling of Theophylline permeation through rabbit skin from different gel formulations at ultrasound intensity 2 W/cm^2.

*Correlation coefficient (r)			
Gel formulations (%w/w)	Model employed		
	Zero-order	First-order	Higuchi-model
HPMC 2%	0.9988	0.9933	0.9827
HPMC 3%	0.9986	0.9921	0.9831
HPMC 4%	0.9985	0.9954	0.9815
Sod. CMC 2%	0.9983	0.9988	0.9832
Sod. CMC 3%	0.9934	0.9984	0.9847
Sod. CMC 4%	0.9947	0.9994	0.9864
Carbopol 1%	0.9922	0.9875	0.9987
Carbopol 2%	0.9891	0.9808	0.9997
Carbopol 3%	0.9654	0.9809	0.9996

*Average of three determinations.

Conclusion

It is possible to enhance the permeation of theophylline through rabbit skin by phonophoresis. The enhancement depends on the nature of the gel base, concentrations and the conditions of ultrasound applications. The optimum condition of ultrasound for maximum theophylline transport is a continuous ultrasound mode with two W/cm^2 intensity. The best transdermal ultrasound gel formulations, for further *in-vivo* study, with a maximum Flux values and optimum rheological properties were 2% HPMC, 4% Na CMC and 2% Carbopol 934P gels.

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