

## A Preliminary Study on the Use of Ultrasound for Obtaining Pulsatile Release

Yelda KOMESLİ, Emel Öykü ÇETİN, Levent KIRILMAZ, Evren GÜNDOĞDU

### ABSTRACT

In this study, the controlled release tablet of diltiazem hydrochloride was developed. The controlled release tablets were prepared with ethyl cellulose by changing the ratio of diltiazem hydrochloride and the in vitro release studies were carried out by using an ultrasonic water bath. According to release results, a new equation was developed that can be used to estimate the release of drug as a function of time and drug content in the formulation. In addition, the effect of ultrasound on the release of diltiazem hydrochloride from controlled

release dosage forms and relationships between release rate constants and drug contents were investigated. This study showed that the developed equation can successfully be used to estimate the effect of ultrasound on the release of drug from the formulations prepared with different concentrations of diltiazem hydrochloride. It was concluded that ultrasound has a very important effect on the release of drug and increases drug release obviously.

**Keywords:** ultrasound; controlled release; pulsatile release

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### INTRODUCTION

Plasma concentrations of drug depend on the frequency of administration. In conventional drug release, the blood concentration of the drug increases, reaches a peak and falls after the drug administration. For these reasons, we plan to work responsive systems in our study. We have examined the response of different drug content of the polymeric tablets to the ultrasound. Polymeric controlled drug delivery systems were used to obtain controlled drug delivery. In this study, the relationship between the release rate constants, ultrasound and drug contents were investigated. It is found that the ultrasound has a very important effect on the release of drug and increases drug release clearly.

The intensive works on responsive implantable polymeric drug systems had begun in 1980's. These systems are categorized in two different groups:

1. Feed-back Controlled Systems (1,2)
  - a. Electrically Controlled Systems (3,4,5)
  - b. Chemically Responsive Systems (6,7)

## 2. Modulatable externally Systems

### a. Magnetically stimulated Systems (8)

### b. Ultrasound Systems (9,10)

The recent researches into the area of controlled drug delivery have been focused on the development of systems which result in either zero-order drug release or a drug release profile which is a simple function of time. This approach has been based on the assumption that optimal clinical outcomes may be achieved by maintaining a constant drug plasma concentration and that drug concentration-effect relationships do not vary overtime. However, it is apparent that varying or pulsed drug release is therapeutically preferable with some drugs, and that there are a number of clinical situations which may benefit from a more responsive approach to drug delivery (9,10).

The use of externally applied ultrasound to trigger release from polymeric delivery devices has been proposed as a means of modulating drug release from implants. These relatively simple devices consist of a polymer and drug and are designed for subcutaneous implantation. A basal level of drug release occurs as a result of diffusion which may be augmented by exposing the area of skin above the implant to therapeutic levels of ultrasound. It has been demonstrated both in vivo and in vitro that release can be significantly enhanced using ultrasound (11,12).

Recently, liposomes in which contains drugs are being used in the drug distribution increased by ultrasound and drug release controlled by ultrasound. Liposomal ultrasound based drug and gen transportation in vivo applications are utilized in the treatment of cardiac disease, stroke and tumor therapy (13). Acoustic energy collection enabled with ultrasound is utilized to activate the drug and gene distribution, and to make changes in tissue transitions (14).

It is investigated that the effects of microbubbles to increasing drug release with ultrasound (15). The other study focuses, characterization and synthesis of polymeric microcapsules on lipophilic drug transportation triggered by ultrasound. Microcapsules consist of a drug-parting depot including various amounts of hexadecane oil with a separate gaseous phase, and a fluorinated end-capped poly (L-lactic acid) shell were made pre-mix membrane emulsification. Under the high diagnostic pressure, microcapsule crashes and gas inside it and lipophilic drug are released (16).

The use of AP-1-conjugated liposomes which include cytotoxic agents followed by HIFU (high frequencies of ultrasound ) pulse ,in the treatment of brain tumor in such a improved way for the distribution of the drugs to the targeted area (17).

In another study, the drug release, loaded to titanium nanotubes from micelles, was enhanced by the application of ultrasound. The effects of ultrasound on the drug release from micelles by applying ultrasound continuously and intermittently. Ultrasound significantly increased the release of drug. The acoustic power which affects the depth of nanotubes describes increased release from polymeric micelles. Long and short waves of ultrasound and 1, 5, 10, 15 pulses per minute applications were examined. The best results were obtained by 15 pulse application in 5 minutes. A linear correlation was found between the number of pulses per minute and the generated thermal energy. Short pulses indicated the great energy and acoustic power. Local drug delivery systems are used in bone infections, fracture healing, secondary bone cancer, tissue repair and reducing inflammation. This type of drug delivery systems can be applied in orthopedic or coronary stents (18-20).

To evaluate the effect of magnetically stimulated drug release, dopamine conjugated iron oxide nanoparticles were designed. Loaded polymeric micelles as drug carriers with titanium nanotube arrays were used to provide stimulus-response drug delivery system. Magnetic stimulated drug release was obtained by adding magnetic nanoparticles in the bottom of nanotubes (21).

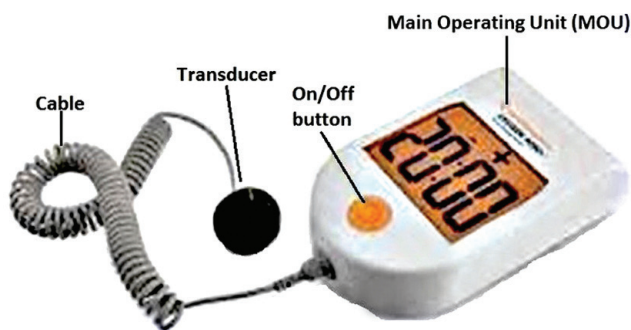
Recent studies show that, the cavitation generated by the ultrasound waves caused to cellular incorporation of macromolecules up to 28 nm. This reversible effect is the result of injury of the plasma membrane created by ultrasound and providing passage halls self-re-sealed in a short time by the cells. Thus non-invasively focused ultrasound can be used for drug and gene delivery to targeted cancer cells and tissues, minimizing side effects, enhancing therapeutic effects, lowering doses of drugs (22).

Acoustically active drugs were developed from encapsulated liposomes which can be activated with ultrasound waves. Due to air contents, liposomes could response ultrasound activity and release drugs. The liposomes were made phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, cholesterol and mannitol. After sonication, lyophilization and rehydration, a hydrating lipid film added in order to make liposomes acoustically active. 1 MHz ultrasound at 2 W/cm<sup>2</sup> for 10 s applied for efficient Ultrasound-triggered release (23).

In another study Doxorubicin activity was enhanced with ultrasound and no effect was observed near tissues without ultrasound in rats (24).

For a long time ultrasound has been applied at hospitals. However, FDA recently, approved the use of Exogen (Exogen is a registered trademark of Bioventus LLC,

Durham, NC 27703 USA. Product No. 81079936 Rev 02012-05) for bone fractures, because of its ultrasonic effects. It can be easily applied by patients at their houses (Figure 1).



**Figure 1.** Photograph and application of Exogen.

(Exogen is a registered trademark of Bioventus LLC, 4721 Emperor Blvd, Suite 100, Durham, NC 27703 USA. Product No. 81079936 Rev 02012-05)

## MATERIALS AND METHODS

### MATERIALS

Diltiazem HCl was supplied from İlsan- İltaş, İstanbul-TURKEY. Ethyl cellulose was purchased from Colorcon Limited, İstanbul-TURKEY. Alcohol (96°) was purchased from Tekel, İzmir-TURKEY. UV-Vis spectrophotometer (Shimadzu 1208), ultrasonic water bath (Elma® Transsonic 460/H, 35 kHz) and oven (Nüve) were also used.

### METHODS

#### UV SPECTRUM OF DILTIAZEM HCl

4 mcg / ml concentration of diltiazem HCl was dissolved in water and the spectrum of this solution detected from 225 to 350 nm against the water. The value of maximum absorbance of the solution ( $I_{max}$ ) was determined.

#### DETERMINATION OF THE SATURATION CONCENTRATION

5.5 g of diltiazem HCl was dissolved in 8 ml distilled water. Mechanical stirring in a water bath at 37 ° C and 150 rev / min stirring speed was kept for 30 minutes. The solution of diltiazem HCl was filtered on paper and 1 ml of the solution was completed to 10 ml with distilled water. Then, 5 ml of this solution also received and complete to 10 ml with distilled water again, absorbance was read in a

spectrophotometer and the concentration was calculated with the help of the calibration equation and the saturation concentration ( $C_s$ ), respectively.

#### SPECTROPHOTOMETRIC DETERMINATION OF DILTIAZEM HCl

100 mg of diltiazem HCl was dissolved and completed to 50 ml with distilled water. 10, 20, 30, 40 and 50 ml samples were taken from the stock solutions respectively and completed to 5 ml with distilled water. The solutions were measured spectrophotometrically at a wavelength of maximum absorbance. Respectively 4, 8, 12, 16 and 20 mg/ml corresponding to absorbance measurement is taken place in the graph, by applying regression analysis " $Y = mX + n$ " was obtained in the form of a linear equation. This quantification was used in all equations.

#### VALIDATION OF ANALYTICAL METHODS

##### Linearity

6 samples obtained by dilution of the stock solutions and their absorbance were read. The linear equation was obtained from the absorbance values and the corresponding concentrations.

##### Accuracy

###### 1. Repeatability

A single 30 mg/ml concentration of diltiazem HCl solution from calibration stock was prepared and measured 6 different times at 234 nm. The absorbance values obtained from these measurements with the help of the calculated concentration of active substance found. Standard deviation and variation coefficient were calculated. Coefficient of variation of less than 2% on proving the reproducibility of the method to be used is a valid approach.

###### 2. Reproducibility

6 samples were prepared from the stock solution at the same concentration (30 mg/ml) and measured at 234 nm. Standard deviation and variation coefficient were calculated. Coefficient of variation of less than 2% on proving the reproducibility of the method to be used is a valid approach.

##### Specificity

In order to prove the analytical method was specific to Diltiazem HCl, the spectrum of alcoholic solutions of ethyl cellulose used in studies was taken in the range of 225-350 nm.

### Stability

25 mL diltiazem HCl solution was taken from calibration stock (100 mg diltiazem HCl / 50ml water) completed with 5 ml of water and measured at 234 nm. The amount of the active substance was calculated accordance to the calibration curve. The stock was stored at 37°C in oven for 4 hours. Absorbance of the solution was measured with the same dilution and calculated. Standard deviations and relative standard deviations were determined of the results.

### PREPARATION OF MATRIX TABLETS

Controlled release matrix tablets containing 25%, 50%, 75% Diltiazem HCl were prepared by using ethyl cellulose. Drug contents of tablets were determined practically. Ethyl cellulose was selected as a polymer to obtain controlled release. Diltiazem HCl and ethyl cellulose were mixed thoroughly in accordance with the general rules of mixing in the mortar. The paste was prepared by using alcohol (96°) such as wetting agent. After that, the paste was passed through an injector (5 mm in diameter) by pressing. It was dried for 30 minutes at room temperature. Then, this cylindrical mass was cut into small tablets with the lancet and they were again dried at 40 °C in an oven during one night. Homogeneity and quantification studies were carried out with the tablets.

### QUANTITATIVE DETERMINATION STUDIES IN MATRIX TABLETS

25%, 50% and 75% active ingredients containing matrix tablets were used. The amount of active ingredient in 3 tablets for each formulation were measured. Each tablet dissolved with 100 ml 96° etil alcohol in the ultrasonic bain. Appropriate dilutions were done and measured their absorbances at 234 nm in UV spectktrofotometre. The amount of the diltiazem HCl calculated according to the calibration curve equation.

### Homogeneity

1 tablet for each formulation was divided into three parts and weighed. Then, each part of tablet dissolved with 96° etil alcohol in the ultrasonic bain. Appropriate dilutions were done and measured their absorbance at 234 nm in UV spectktrofotometre. The amount of the diltiazem HCl calculated according to the calibration curve equation. Standart deviation and relative standart deviations were calculated.

### IN VITRO RELEASE STUDY BY USING APPLYING ULTRASOUND

Tablets, which have three different drug contents, were placed in 100 ml distilled water and the release studies were carried out by using an ultrasonic water bath (Elma® Transsonic 460/H, 35 kHz). For every formulation ultrasound was applied intermittently at pre-determined times. The amounts of drug were determined spectrophotometrically (234 nm) by applying with or without ultrasound. Release profiles were plotted as a function of time. The temperature of the release medium was remained constant at 37 °C during the release studies.

### RESULTS

#### UV SPECTRUM OF DILTIAZEM HCl

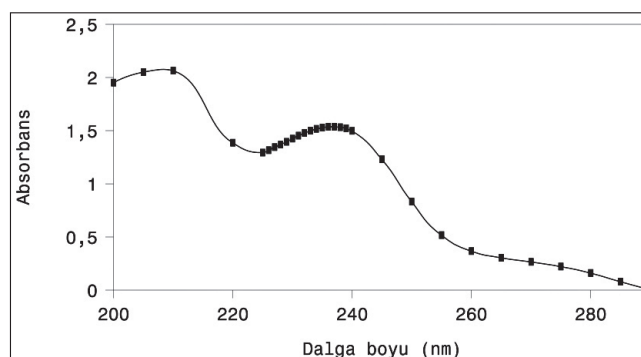


Figure 2: The spectrum of diltiazem HCl in the distilled water

#### Linearity

$$r^2 = 0.9997$$

$$y = 20.6x - 3.0 \quad (y = \text{kons.}; x = \text{abs.}) \quad (\text{Equation 1})$$

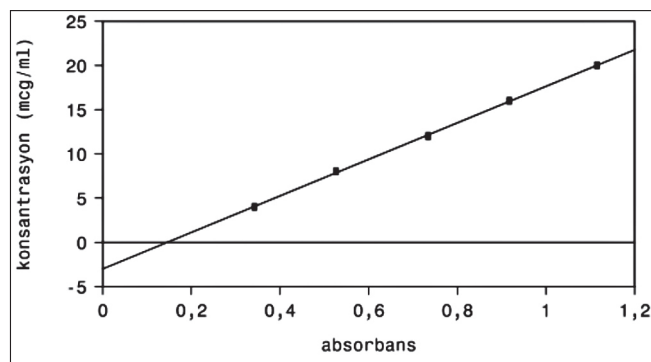


Figure 3: The calibration curve of diltiazem HCl

## Repeatability

**Table 1.** The results of repeatability

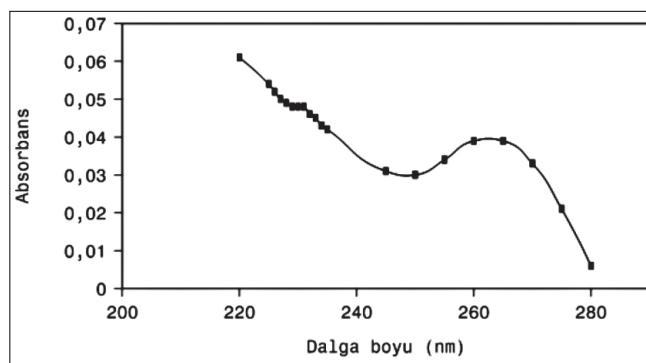
Sample (30 µl/5 ml)	Absorbance	Concentration (µg/ml)	Standart Deviation	Relative Standart Deviation (%)
1	0.692	11.282	0.03	0.27
2	0.692	11.282		
3	0.693	11.302		
4	0.694	11.323		
5	0.693	11.302		
6	0.696	11.364		

## Reproducibility

**Table 2.** The results of reproducibility

Sample (30µl/5 ml)	Absorbance	Concentration (µg/ml)	Standart Deviation	Relative Standart Deviation (%)
1	0.692	11.282	0.07	0.65
2	0.686	11.158		
3	0.684	11.117		
4	0.692	11.282		
5	0.685	11.137		
6	0.689	11.220		

## Shecifty



**Figure 4:** UV spectrum of the ethyl cellulose

## Stability

**Table 3.** The results of stability

Sample 25 µl/5 ml (=10 mcg/ml)	Practical drug content	Drug content at 37°C	Standart Deviation	Relative Srtandart Deviation (%)
1	9.465mcg/ml	9.444mcg/ml	0.015	0.157

## THE FORMULATIONS OF MATRIX TABLETS

**Table 4.** The formulations of matrix tablets

Drug content in the formulations	Diltiazem HCl (mg)	Etil cellulose (mg)
% 25	125	375
% 50	250	250
% 75	375	125

## QUANTITATIVE DETERMINATION STUDIES IN MATRIX TABLETS

**Table 5.** The results of quantitative determination studies

Formulations	Practical drug content (%)	Average drug content (%)	Standart Deviation	Relative Srtandart Deviation (%)
% 25	24.53	24.40	0.194	0.79
	24.18			
	24.5			
%50	46,92	46,897	0,025	0,054
	46,87			
	46,9			
%75	69,12	69,493	0,333	0,479
	69,6			
	69,76			

## HOMOGENEITY

**Table 6.** The results of homogeneity studies

Formulations	Mass (mg)	Diltiazem HCl (mg)	Diltiazem HCl (%)	Standart Deviation	Relative Standart Deviation (%)
% 25	6.0	1.246	20.77	0,251	1,199
	4.5	0.94	20.88		
	2.4	0.51	21,25		
% 50	5.2	2.077	39.95	0.076	0,191
	3.8	1.520	40		
	4.8	1.925	40.1		
% 75	2.4	1.60	66.7	0,624	0,924
	4.4	2.916	66.27		
	2.4	1.62	67.5		

**IN VITRO RELEASE STUDY BY USING APPLYING ULTRASOUND**

The release profiles of diltiazem HCl in ultrasound applying are shown in Fig. 5-7. It was seen that ultrasound played an important role in controlling diltiazem HCl release and a pulsatile release function was observed. When the ultrasound was applied in interval time, the release of diltiazem HCl was more than without ultrasound applying. The release rates of all tablets are given in Table 7. The release rate was increased during ultrasound applying.

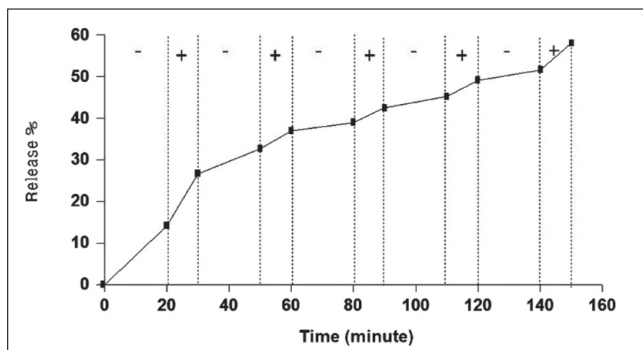


Figure 5. The release results by applying intermittently ultrasound for formulation of 25% diltiazem HCl.

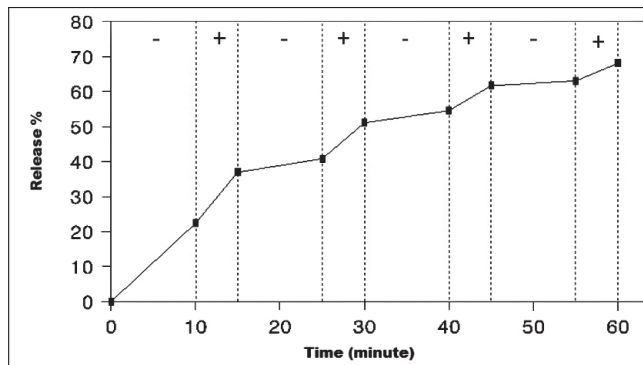


Figure 6. The release results by applying intermittently ultrasound for formulation of 50% diltiazem HCl.

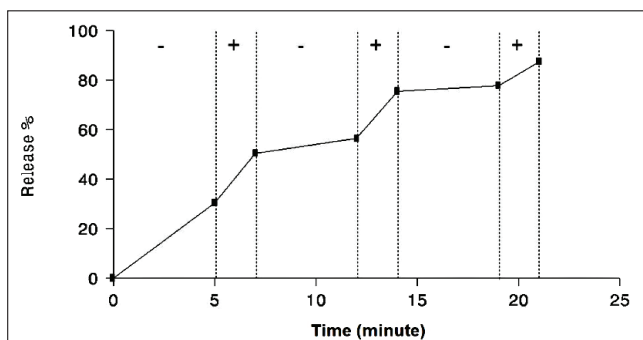


Figure 7. The release results by applying intermittently ultrasound for formulation of 75 % diltiazem HCl

Table 7 The release rates of formulations that have different drug contents by applying intermittently ultrasound.

Practical Drug Contents of Formulations (%)	24.9 %	48.5 %	72.8 %
Average Release Rates (k) (min. <sup>-1</sup> ) (applied ultrasound)	0.677	1.49	9.775
Average Release Rates (k) (min. <sup>-1</sup> ) (not applied ultrasound)	0.120	0.368	0.825

The correlation between drug contents and average release rates, which were given at Table 7, was investigated and the best correlation was found as shown in Figure 7.

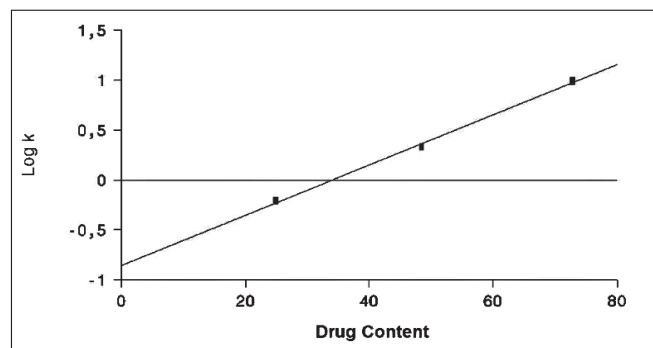


Figure 8. The correlation of release rates and drug contents by applying intermittently ultrasound.

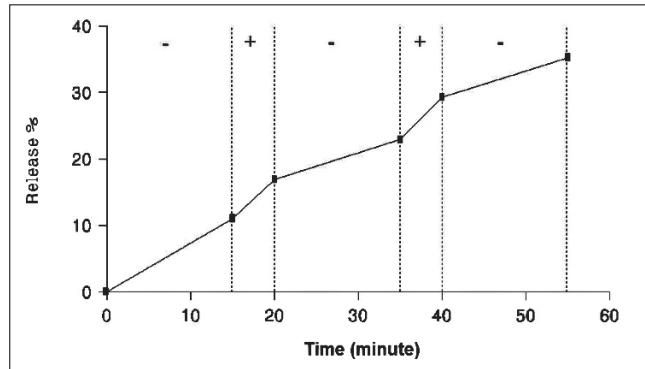
The equation of the correlation above is:  
 $\text{Log } k = 0.00251 \text{ drug content} - 0.853$  (Equation 2)

**THE VALIDATION OF EQUATION**

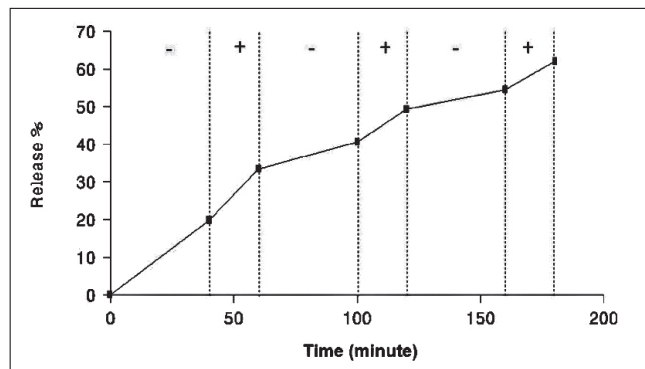
Controlled release matrix tablets containing theoretically 15% and 35% diltiazem HCl were prepared for this study. The practical drug contents of these formulations were found 13.4% and 34.3%, respectively. Ultrasound was applied intermittently. At pre-determined times determinations were made spectrophotometrically for two formulations. Release profiles were plotted as a function of time and showed in Fig 5-6. It was shown that a pulsatile release was obtained by intermittently applying ultrasound to the controlled release tablets containing drug (Figure 5-7). When ultrasound was applied, the release rates were increased definitely (Table 8). A desired formulation can be prepared with the aid of correlation between release rates and drug contents (Eq. 2) (Figure 9-10).

**Table 8.** The release rates of formulations that have 34.5% and 13.4% diltiazem HCl.

Drug Contents of Formulations (%)	Theoretical		Practical	
	34.3 %	13.4 %	34.3 %	13.4 %
Average Release Rates (k) (min. <sup>-1</sup> )	1.02	0.304	1.23	0.405



**Figure 9.** Applying intermittently ultrasound for formulation of 34.3 % diltiazem HCl.



**Figure 10.** Applying intermittently ultrasound for formulation of 13.4 % diltiazem HCl.

## CONCLUSION

Results are prove that a pulsative release system can be obtained by ultrasound from matrix systems including diltiazem HCl. Release velocity is increased considerably when ultrasound applied. This increase is about 5 times greater in formulations including %25 and % 50 active substances, and it became 12 times greater in the formulations including % 75 active substances. This situation verified our expectations about the effect of ultrasound on the release velocity would be much higher after a known substance value. Having the releases in zero order or at a constant velocity has been an advantageous position. Thus guessing in advance the time interval of the release of a complete drug in formulations would become possible.

By this method, the amount of the drug to be released from the delivery system which is implemented by adjusting the ultrasound application time interval can be safely utilized. This situation can be easily submitted to the doctor as a written info-note on formulation (ie. Which amount of drug dosage will be released at how many seconds ultrasound applied). Ultrasound which finds application areas mostly in hospitals nowadays along with the improved technology has become easily implicated to the patients in their houses. This draws our attention as a lately published article in a medicine periodical. FDA has approved the use of instrument Exogen which utilizes ultrasound on the treatment of fractures. In near future, patients can apply ultrasound by their selves whenever they needed, without depending on the doctors and hospitals, by this miniature ultrasound instrument. It was concluded that ultrasound may have a important effect on the release of drug and increases drug release clearly and thought that such an apparatus can also be used for obtaining a pulsatile release both at hospital and home.

## Pulsatil Salm Elde Edilmesi Amacıyla Ultrason Kullanımı Üzerine Bir Ön Çalışma

### ÖZET

Bu çalışmada, diltiazem hidroklorürün kontrollü salım tableti geliştirilmiştir. Kontrollü salım tabletleri diltiazem hidroklorürün değişen oranlarında etil selülozla hazırlanmış ve in vitro salım çalışmaları ultrasonik su banyosu kullanılarak gerçekleştirilmiştir. Salm sonuçlarına göre, formülasyonun ilaç içeriğini ve zamanın bir fonksiyonu olarak ilaç salımını tahmin etmede kullanılabilecek yeni bir denklem geliştirilmiştir.

Kontrollü salım dozaj şekillerinden diltiazem hidroklorürün salımına ultrasonun etkisine ilave olarak, hız sabiti ve ilaç içeriği arasındaki ilişkiler araştırılmıştır. Bu çalışmada geliştirilen denklem, farklı konsantrasyondaki diltiazem hidroklorür ile hazırlanmış formülasyonlardaki ilaç salımında ultrasonun etkisini hesaplamak amacıyla kullanılabileceğini başarılı bir şekilde göstermiştir. Ultrasonun ilaç salımında çok önemli bir etkiye sahip olduğu, ve ilaç salımını açık şekilde arttırdığı ispatlanmıştır.

**Anahtar kelimeler:** ultrason; kontrollü salım, pulsatil salım

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