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A PRELIMINARY INVESTIGATION ON THE ESTIMATION OF THE SUSTAINED-RELEASE OF INDOMETHACIN FROM AGAR BEADS PART I

AGAR MİKROKÜRELERDEN İNDOMETAZİNİN SÜREKLİ SALIM HIZININ TAHMİN EDİLMESİ ÜZERİNE BİR ÖN ÇALIŞMA

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In this study agar beads containing indomethacin were prepared at different polymer-drug ratios. It was found that the release rate of indomethacin from agar beads was inversely related to the drug content. Lactose was added to the formulations in order to investigate its effect on the release of indomethacin from agar beads. Lactose increased the release rate of indomethacin and in vitro release studies exhibited a √t dependence indicating a diffusion controlled process from a matrix formulation. All of the prepared formulations gave prolonged release. The release profiles of the formulations were compared with Indocid-R available in the market. The release criteria of all the formulations and Indocid-R were examined according to USP criteria given for indomethacin and general release criteria. At the end of the study, the obtained data were evaluated mathematically. Two equations to be used in the preparation of agar-indomethacin beads with or without lactose for optimum formulation were estimated. It was found that the estimated equations were in good agreement with the observed release results. In addition, in this study the in vivo distribution of agar beads prepared with barium sulfate as a contrast substance in the gastro-intestinal tract was also examined.

Bu çalışmada indometazin içeren agar mikroküreler farklı polimer-ilaç oranlarında hazırlandı. Agar-mikroküre lerden indometazin salımının ilaç içeriğiyle ters orantılı olduğu bulundu. Formülasyonlara değişik oranlarda laktoz ilave ederek agar mikroküre lerden indometazinin salımına etkisi incelendi. Laktozun ilave edilme oranına bağlı olarak salım hızını arttırdığı gözlendi. Matriks yapıdaki formülasyonlardan ilaç salımlarının difüzyon kontrollü bir şekilde √t kinetiğine uygun olarak gerçekleştiği saptandı. Hazırlanan tüm formülasyonlarda uzatılmış bir salım elde edildi. Elde edilen salım profilleri, indometazinin piyasada mevcut bulunan Indocid-R preparatı ile karşılaştırıldı. Salım sonuçları Amerikan Farmakopesinin indometazin için verdiği salım kriterleri ile ve sürekli etkili preparatlar için genel olarak verilen salım kriterleri ile karşılaştırıldı. Çalışma sonunda elde edilen veriler matematiksel olarak değerlendirildi. Laktoz içeren ve içermeyen agar mikroküre lerden indometazinin salım hızını tahmin ederek, gerekli polimer-ilaç oranlarının hesaplanmasına olanak verecek şekilde iki denklem elde edildi. Elde edilen bu denklemlerin pratik olarak gözlenen sonuçlarla çok iyi bir korelasyon gösterdiği bulundu. Ayrıca radyoopak madde olarak baryum sülfat ilave edilerek hazırlanan agar mikroküreler kullanılarak, agar mikroküre lerin in vivo olarak dağılımları ve mide-bağırsak geçişleri röntgenografi tekniği ile incelendi.

Keywords : *Agar beads; Dissolution test; Sustained-release ; Formulation optimisation; Indomethacin; İn vivo distribution*

Anahtar kelimeler: *Agar mikroküreler; Çözünme hızı testi; Sürekli salım; Formülasyon optimizasyonu; İdometazin; İn vivo dağılım*

Introduction

In order to prolong the drug action and to avoid excessive drug concentrations in plasma and tissues the use of synthetic membranes and some natural polymers for controlled release of bioactive compounds has recently been investigated. For example, agar, agarose and alginate beads were used for encapsulation purposes. These materials form gels when used in adequate concentration upon cooling to about 40°C and they remelt on being heated to about 85°C (1-3). Nakano et al showed (4-6) in their studies that agar prepared from various species of Gelidium and other red algae which belong to the Rodophyceae could be used successfully in controlled release dosage form design. They reported that agar beads were suitable as a vehicle for sustained release dosage forms. In another study different concentrations of

* Correspondence

agar solution were used in the preparation of agar beads contaning phenobarbitone sodium. The results of dissolution study indicated that agar beads could be useful for the preparation of sustained release dosage forms. It was found that the Higuchi kinetic model for dissolution described fully the pattern of dissolution found under the preparative conditions(7).

In recent years, optimization techniques have become more widely used in the pharmaceutical industry. The responses from the predictive models are then used for optimization. Once experimental data are collected and relationships generated by regression analysis, the formulator is able to select the best formulation. The results of an optimization study, especially the graphic output, can enable for product improvement (8, 10).

Indomethacin is used as a non-steroid anti-inflammatory drug for arthritis which is usually administered orally in a conventional and retard capsule form. Conventional dosage form results in side effets on gastrointestinal and central nervous system in some patients. The severity of these side effects seems to be related to the high initial plasma concentration. It is probable that a sustained-release dosage form would reduce the severity of these side effects (11,13). Sustained-release dosage forms in the form of suppositories and calcium alginate gel beads of indomethacin were prepared and their absorptions were investigated. The maximum concentration and area under the time curve of plasma concentration of indomethacin were found lower than those of indomethacin powder or commercial suppositories (14,15).

In the present study a possible use of agar for sustained-release of indomethacine and the effect of lactose (as a water soluble excipient) on the release of indomethacin were examined and two equations were estimated in order to use in the formulation optimisation of agar-indomethacin beads with or without lactose. Three-dimensional response-surface graphs for percentage of indomethacin released as a function of percentage of drug content and dissolution time were generated. In vivo distribution and gastric emptying agar beads were examined with X-ray.

Materials and Methods

1-The preparation of agar beeads

2% agar solutions were prepared. For this purpose, water was added to powdered agar (400 mg) in an erlenmeyer flask to make a 20 ml solution and the suspension was heated up to about 95°C until the agar was completely dissolved. Indomethacin (<75μ) was suspended in the agar solution and stirred at 900 rpm for homogenisation. The drug suspension was taken into a dropper (diameter of 1.1 mm) and dropped onto the top of paraffine solvent (+4°C) which was cooled by immersion in ice-water. Agar beads were formed and solidified when the drug supension came into contact with the cold paraffine solvent (Fig.1). Beads were separated from the solvent by filtration and washed with ether twice for removing residual paraffine. Then the agar beads obtained in this way were dried at 50°C in an oven. The dried agar beads were separated into size fractions using standard sieves (Retsch sieving system).

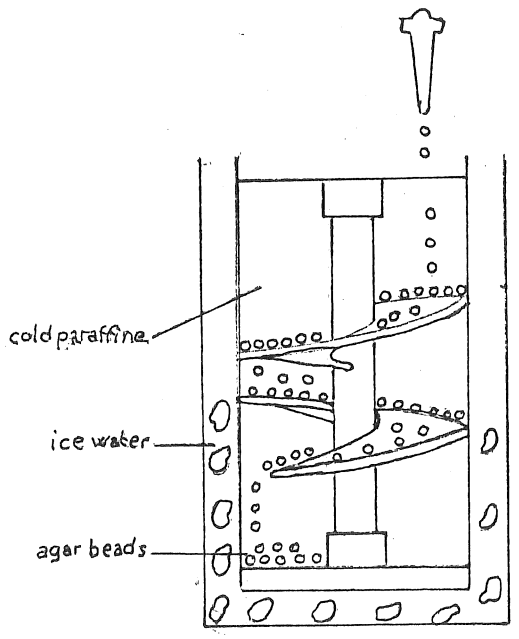

Fig.1. The schematic representation of the preparation of agar beads

2- Variation of formulation

2.1. Three different agar-indomethacin ratios (2:1, 3:1 and 4:1) were used to determine the effects on physical characteristics and dissolution properties of the beads.

2.2. By adding lactose to the formulations at different ratios, it was aimed to investigate the effect of lactose on the release rate. For this aim, the agar beads were prepared at 1:0.5:1, 1:1:1 and 1:3:1 agar-lactose-indomethacin ratios.

All the formulations were prepared in the form of three bathces.

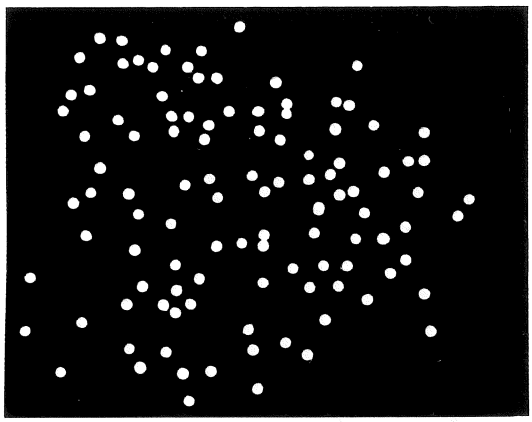

Fig.2. The photomicrograph of the agar beads after drying process.

3- Assay procedure

The total drug content of the beads was determined by dissolving accurately the weighed amount of the beads prepared at each polymer-drug ratio in 100 ml of pH 6.2 phosphate buffer (16) which was heated up to about 95°C and observing the spectrophotometric absorbance at 318 nm. It was established that the Beer's law was followed and the agar did not interfere with the assay. Triplicate samples were assayed and the mean values were reported.

4- In vitro dissolution studies

The dissolution tests were carried out in standard USP dissolution beakers containing 500 ml of pH 6.2 phosphate buffer and 37°C 0.5°C using rotating paddle. Tween 80 (0.02%) was added to the dissolution fluid to overcome the poor wettability of indomethacin (17,18). The dissolution fluid was stirred at 100 rpm. Accurately weighed 25 mg of indomethacin powder (<75μm) and the agar beads which were calculated to contain 25 mg indomethacin were used for dissolution studies. Five ml. aliquots were taken at predetermined times up to 24 hours. These aliquots were spectrophotometrically assayed directly after filtration and returned to the beakers. Each determination was carried out in triplicate and the mean values were used. At the end of the measurements of 24 hours, the amount of indomethacin remained in the agar beads was determined.

5- Mathematical evaluations

In order to explain the variation in % release of indomethacin from agar beads with and without lactose, a multiple linear regression model has been assumed. This model is in the form of;

$$y = \alpha + \beta_1 x + \beta_2 \log t + e \qquad \text{(Eq.1)}$$

where, y is the observed % release of drug, e is the error term. α, β_1 and β_2 are the coefficients to be

Table 1 : The effect of polymer ratio on drug loading

Agar-indomethacin ratio	Expected drug content (%)	Mean observed drug content (%)
1:0.5	33.3	32.75
1:0.25	20	18.1
3:1	25	23.87

Table.2. The effect of polymer and lactose ratios on drug loading

Agar-lactose: indomethacin ratio	Expected drug content (%)	Expected lactose (%)	Mean observed drug content (%)	Mean observed lactose (%)
1:0.5:1	40	20	35.52	21.49
1:1:1	33.3	33.3	30.57	34.72
1:3:1	20	60	18.84	60.87

estimated. x and $\log t$ are % content of drug and logarithm of time, respectively.

Minitab Release 5.1 statistical analysis package has been used for statistical evaluation of the experimental data.

6. In vivo studies

The formulation of 1:2 agar-drug ratio was prepared for examining gastric emptying and in vivo distribution of agar beads. Barium sulfate was incorporated into the formulations to replace the drug as a contrast substance. The agar beads containing barium sulfate (100 mg) were filled into a hard gelatin capsule (size:4). The capsule was administered to a healthy volunteer (age:25 and weight:76 kg) after breakfast with ≈ 200 ml of water. Then, agar beads was followed for about 8 hours by taking X-ray photographs.

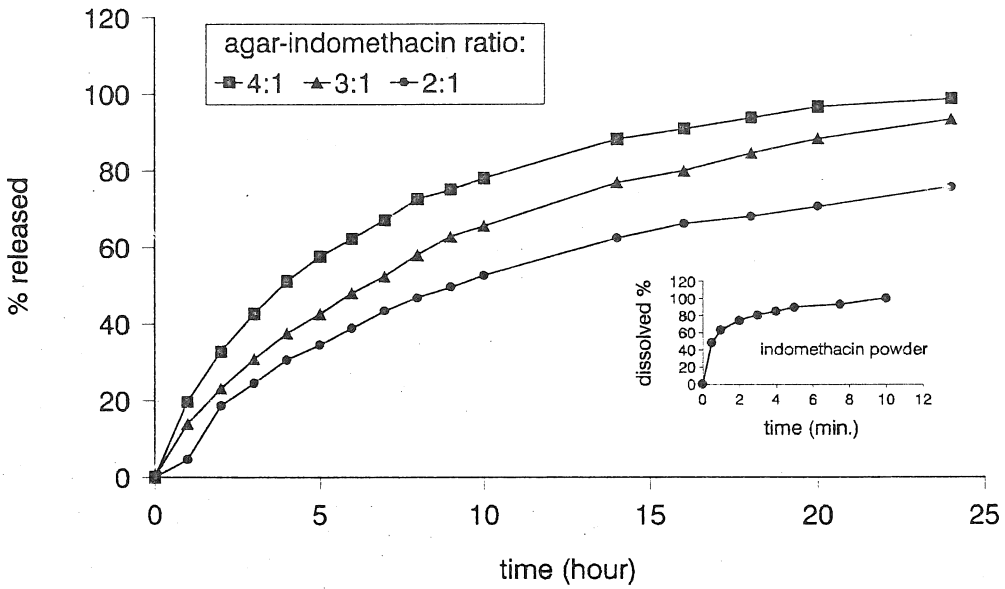

Fig.3. The release of indomethacin from agar beads prepared at different polymer-drug ratios (insert: The dissolution profile of the indomethacin powder).

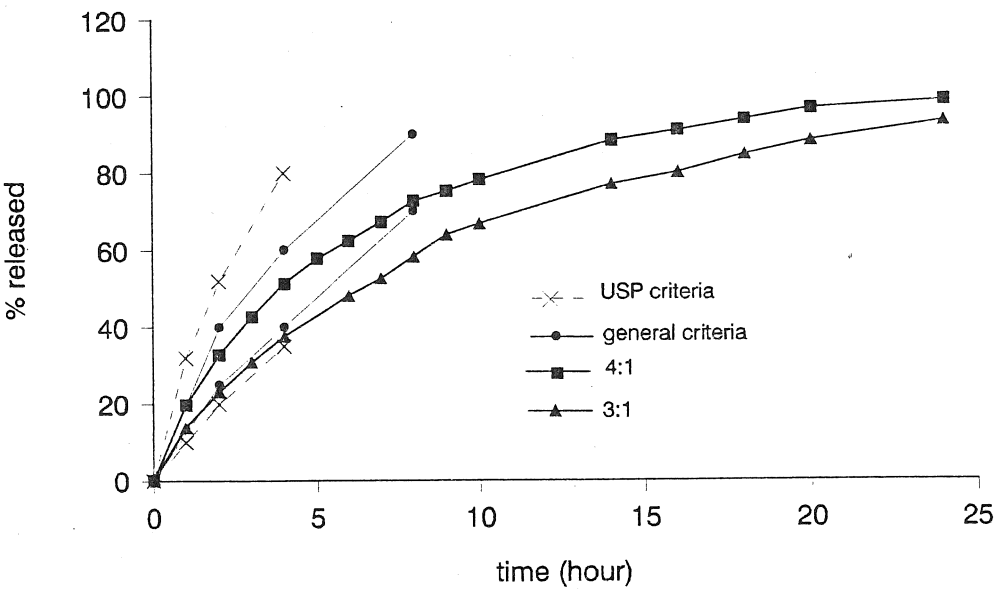

Fig. 4. The comparison of the release of indomethacin from the formulations of 4:1 and 3:1 with two release criteria

Results and Discussion

1. Effect of formulation variables on physical properties

The shapes of the agar beads obtained were generally spherical with an initial diameter ranging from 3 to 3.5 mm. After drying process, beads of 1.14-1.5 mm in diameter obtained by sieving were used in dissolution tests of the formulations. When the amount of indomethacin was increased in agar beads, the shapes of the beads became more regular and spherical. The results related with drug loading are given in Table I and II.

2. Effect of the formulations on the release rate of indomethacin

2.1. The effect of the formulations prepared without adding lactose on the release of indomethacin

It was observed that the dissolution rates are inversely related to the indomethacin percentage in the agar beads since the contact surface of indomethacin in agar beads is comperatively smaller at higher drug contents. Thus, lower dissolution rates were obtained. The cumulative percentage of the released indomethacin was plotted as a function of time in Fig.3. All the agar beads containing indomethacin have released the drug much more slowly than the indomethacin powder.

It is generally known that the desired release rates for 12 hr sustained-release preparations are in the range of 25-40% by 2 hrs, 40-60% by 4 hrs, and 70-90% by 8 hrs (19). Furthermore, as indicated in USP XXII, there are some limitations for the release of indomethacin from extended-release dosage forms for a dosing period of 12 hour. The releases are also in the range of 10-32% by 1 hr, 20-52% by 2 hrs and 35-80% by 4 hrs and not less 60% by 12 hrs. (16). It can easily be seen from Fig. 4 that only the 4:1 formulation has satisfied both the general sustained-release criteria and USP criteria. When the pharmacopoieal criteria were also taken into consideration, it was observed that the ones with the drug ratios of 4:1 and 3:1 could be accepted as suitable formulations. In vitro release rate of a commercially available retard formulation of indomethacin (Indocid-R 75 mg) was higher than any of the prepared formulations. In addition, as shown in Fig.5 the amount of indomethacin released from Indocid-R was higher than the two release criteria mentioned above. In this case, it should be considered that toxic levels may be reached and advers reactions may be observed.

When the percent released amount of indomethacin is plotted against the square root of time, it has been observed that the

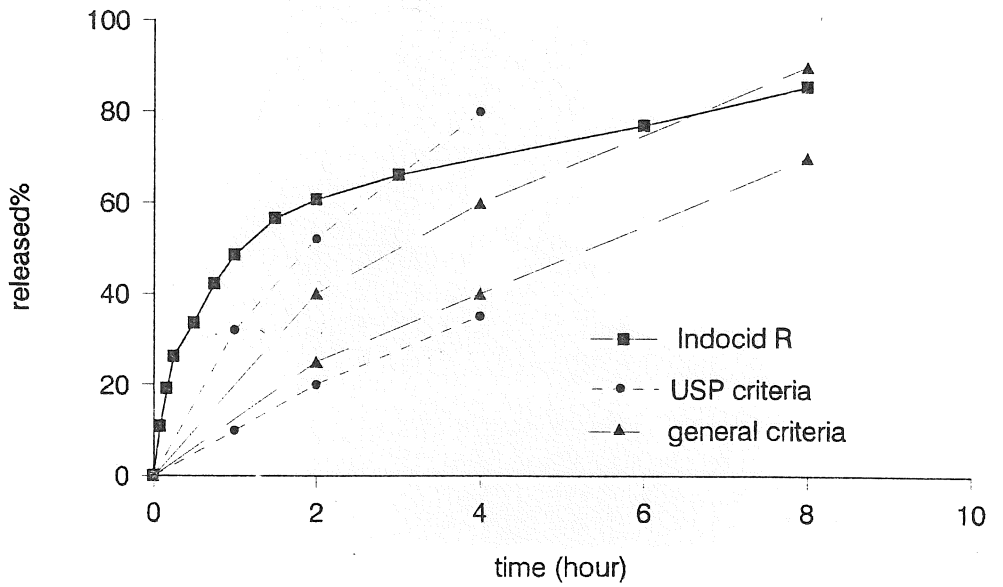

Fig.5. The comparison of the release-rate of Indocid-R with two release criteria

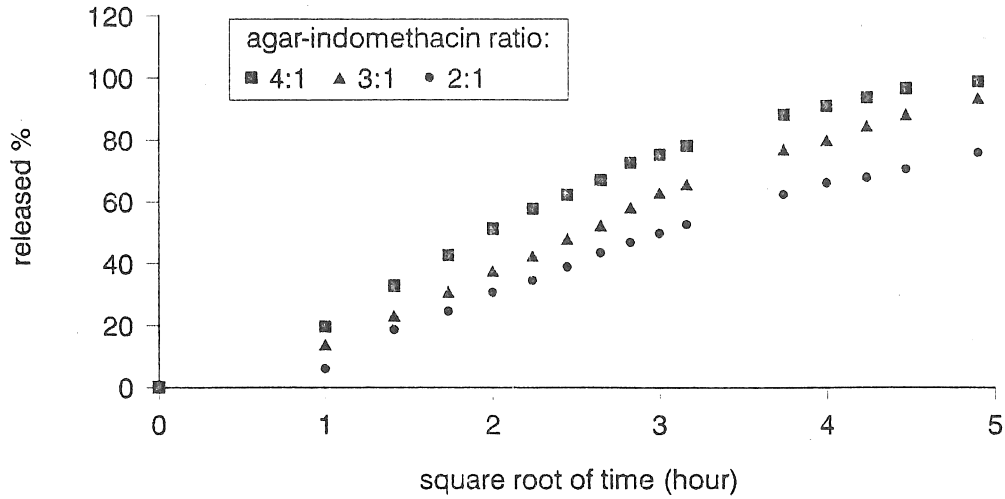

Fig. 6. The release of indomethacin as a function of square root of time for three different drug contents

relationship was approximately linear for the first 3 hours (Fig.6). Deviations from the straight line during the latter period of release may be attributed to the accumulation of the drug in the release media (non-sink condition) and the theoretically expected deviation from linearity as the fraction of the drug released increases (20,21). Also, it is known that the matrix systems generally do not display zero-order release kinetics. The release rate from a planar matrix is usually proportional to the square root of time. Similar observations were made earlier in the drug release from compressed hydrophilic matrices (7,22,23). We have observed in the present study that the release rates from agar beads are also proportional to the square root of time. Non-zero order release in matrix systems can

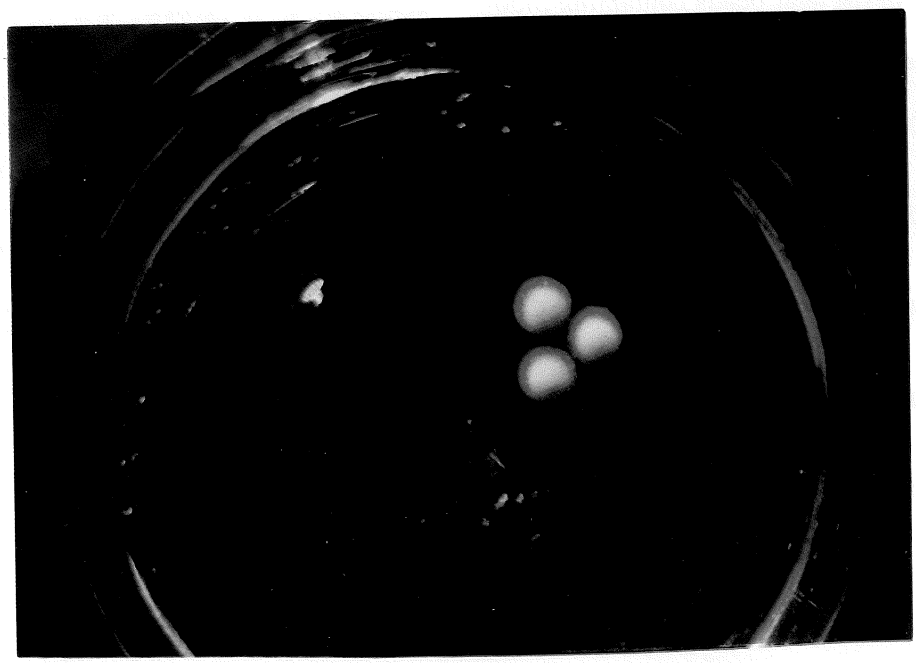

Fig.7. The photomicrograph of the formation of clear zone around the agar beads cotaining indomethacin during dissolution test

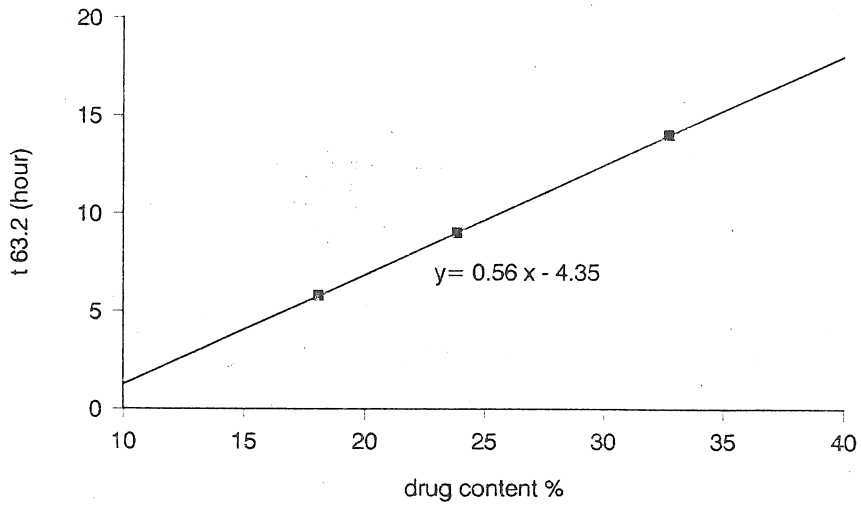

Fig.8. The in vitro $t_{63.2}$ values as a function of percent indomethacin content

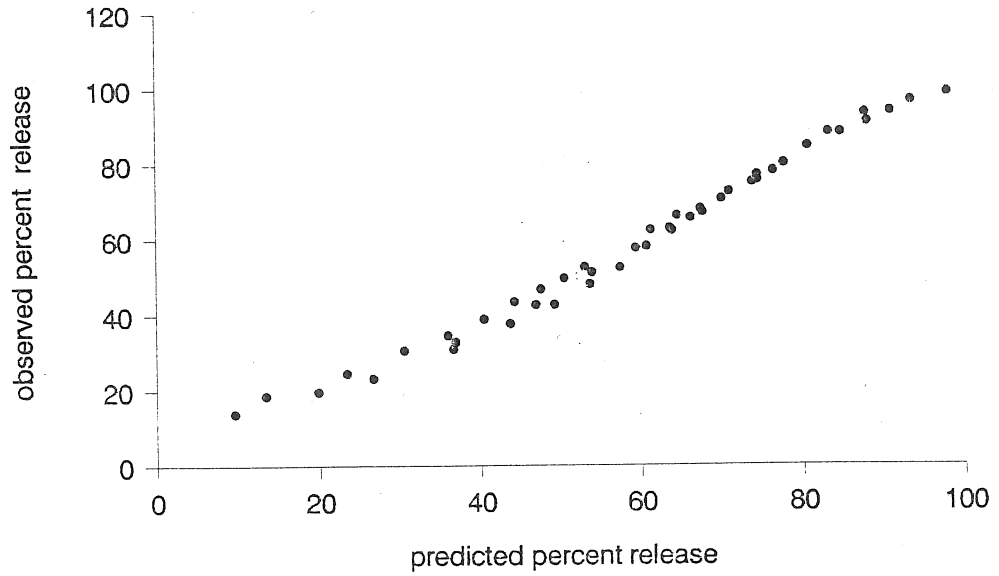

Fig.9. The scatter diagram of the predicted and observed release percentages obtained from agar beads prepared without lactose

be attributed to the changing distance since the drug must travel from the interior of the matrix to the matrix surface. It is expected that the release rate decreasesas this, diffusinal distance increases with time. Therefore, the zero-order release could not be obtained here due to this matrix formulation. The release rate related inversely with the indomethacin content since the contact surface for dissolution of indomethacin was increased. In this case, water penetrated into the beads, hydrating the polymers and also dissolving the drug and then diffused out through the swelled beads. The swelling of polymers in the dissolution medium increased the porosity which caused higher release rate. A clear annular zone appeared during dissolution studies as the drug dissolved and diffused from the matrix (Fig.7). The spherical bead remained intact but swelled to approximately two times of its original diameter during the course of the dissolution experiments.

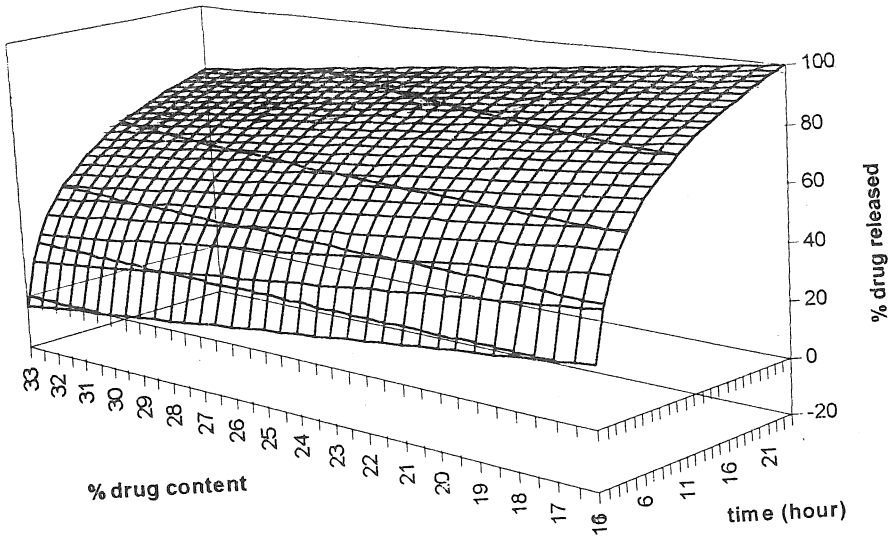

Fig.10. The response-surface graph for percentage indomethacin released from the formulations without lactose as a function of percentage drug content and dissolution time

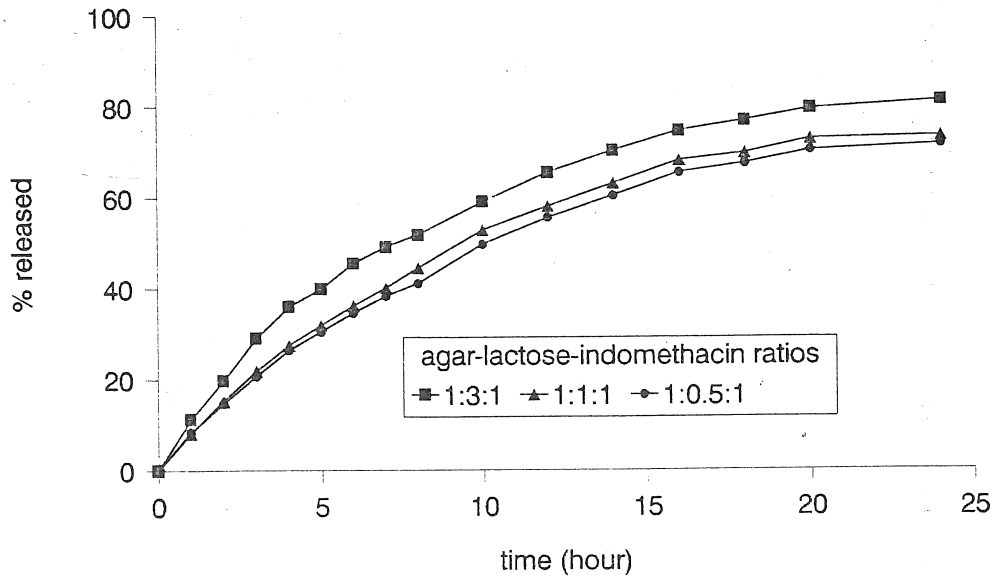

Fig.11. The release of indomethacin from the agar beads containing lactose

The $t_{63.2}$ value is defined as the time of 63.2% of the drug to be dissolved, and it has been suggested as the best in vitro variable in relation with in vivo activity. The plot of $t_{63.2}$ values as a function of the content of indomethacin is given in Fig.8 in which an approximately linear relationship was observed.The results indicated that polymer-drug ratio was effective on the release rate of indomethacin.

The dissolution results obtained until 24 hours were evaluated mathematically. For this aim, the multiple regression model given in Eq.1 was fitted. The estimated model which is in the form of was found to be the best

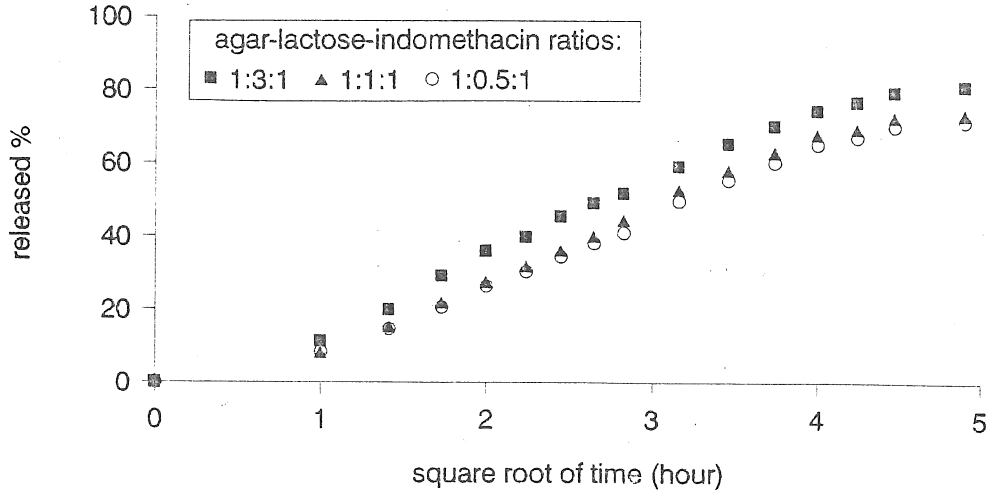

Fig.12. The release of indomethacin as a function of square root of time for three different lactose contents

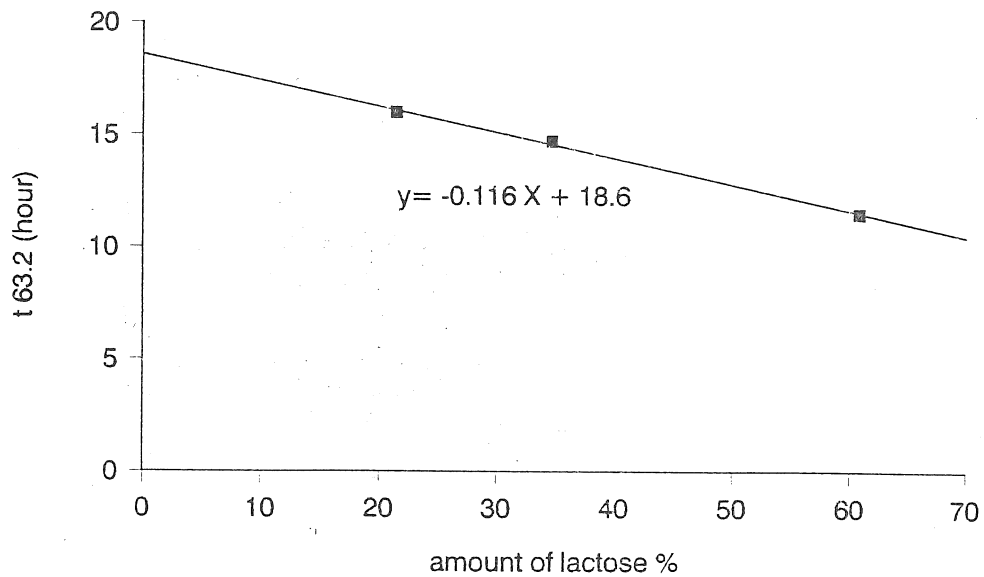

Fig.13. The in vitro $t_{63.2}$ values as a function of the amount of lactose added to the formulations.

$$y = 45.2 - 1.49x + 56.6 \log t \qquad \text{(Eq.2)}$$

fitted one for % release of drug from agar beads without lactose ($F=2961$; 5 $p<0.001$) with the determination coefficient of 97.8%. Following scatter diagram obtained by plotting the fitted values against the observations indicates how well the fitted values agree with the observed ones ($r^2=0.985$) (Fig.9). A three-dimensional response-surface graph for percentage of indomethacin released as a function of percentage of drug content and dissolution time was generated by using Eq.2 in order to enable selection of optimum formulation without lactose (Fig.10).

2.2. The effect of lactose added to the formulations on the indomethacin releaese

Lactose was used as a water soluble substance in order to speed up the release of indomethacin from agar beads by adding to the formulations at different ratios as given in Table 2. It was observed that the release of indomethacin increased proportionally as the amount of lactose increased. Dissolution rates of these formulations are given in Fig.11.

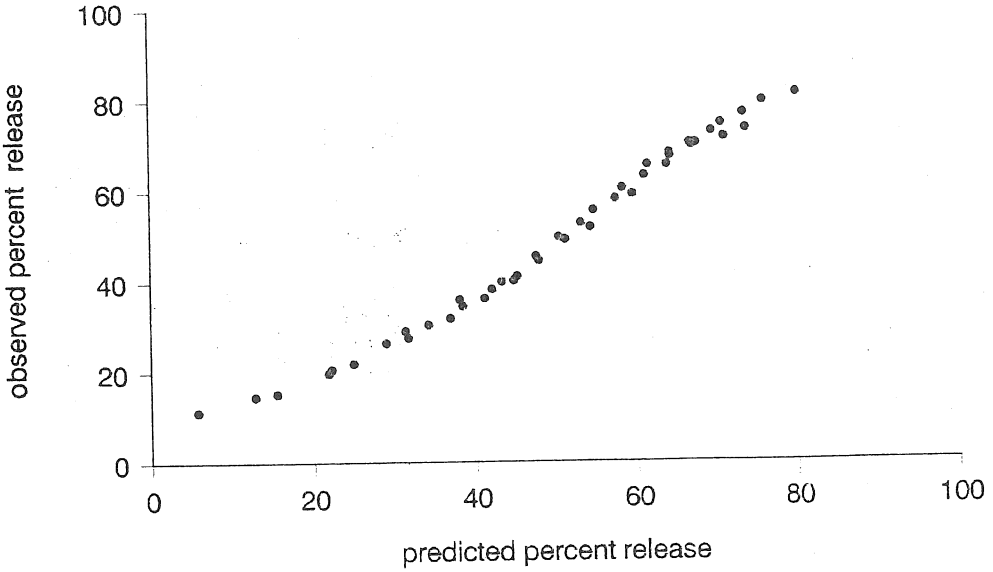

Fig.14. The scatter diagram of the predicted and observed release percentages obtained from agar beads prepared with lactose

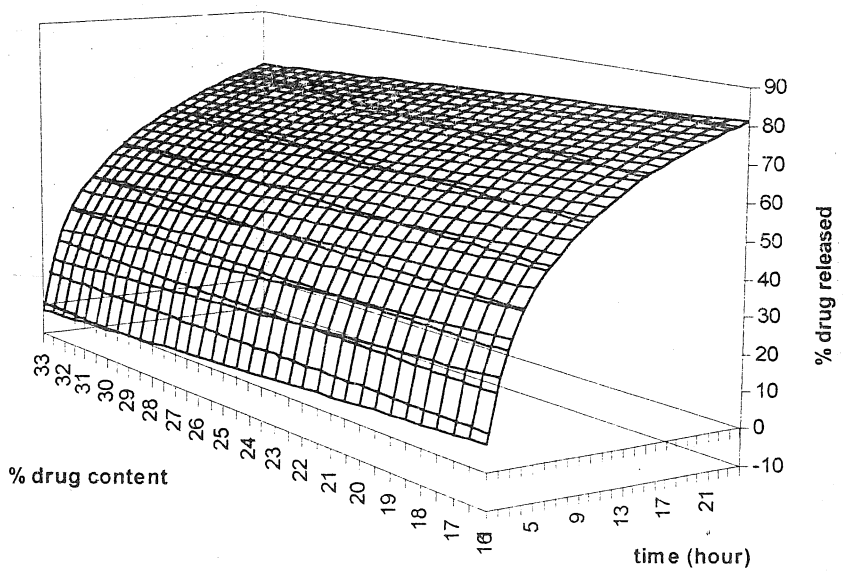

Fig.15. The response-surface graph for percentage indomethacin released from the formulations with lactose as a function of percentage drug content and dissolution time.

The quadratic nature of the relationship may be due to the changes in the porosity of the matrix after dissolution at higher lactose content. In other words the contact surface of indomethacin with the dissolution medium increases as the content of lactose increases. But zero-order release could not be obtained as expected in matrix systems. Release kinetic was also investigated for all beads by plotting the dissolution results against square root of time. An approximate linear relationship was observed especially in the earlier stages as seen in Fig.12.

The results obtained for the relationship

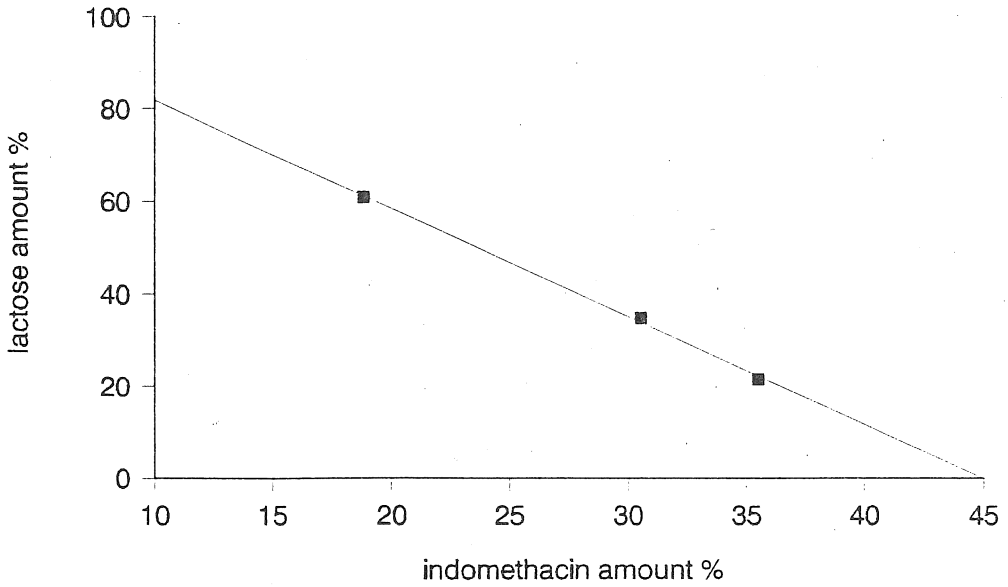

Fig.16. The relationship between the indomethacin and lactose amounts in the formulations

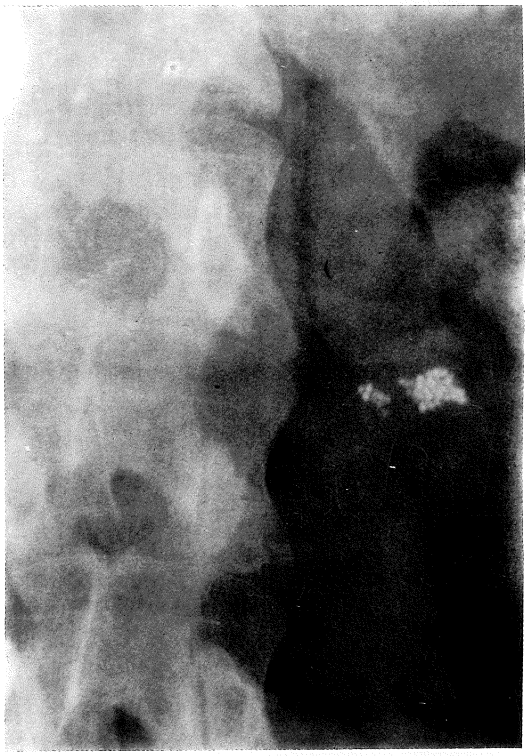

Fig.17-a. X-ray photographs of agar beads containing barium sulfate as a contrast substance in the gastro-intestinal tract (5 min.)

between the $t_{63.2}$ values and the lactose content reflected similar conclusions. Fig.13 has indicated that the $t_{63.2}$ values decreases as the lactose content in the formulation increases.

In order to investigate the effect of the drug content and the time on the % drug release of the formulations prepared with lactose, multiple regression model given in Eq.1 has been fitted similar to the one obtained for formulations without lactose. The estimated model which is in the form of

$$y = 16\text{-}0.547x + 53.9 \log t \qquad \text{(Eq.3)}$$

was found to be the best fitted model for % release of drug with lactose ($F=2116.1$, $p<0.001$). The coefficient of determination was 0.968 for this model. By using this equation, % release of indomethacin from the formulations containing lactose can be predicted. Almost all the predicted values were in good agreement with the observed ones similar to the results obtained for the experiment without lactose($r^2=0.983$) (Fig.14). A three-dimensional response-surface graph for percentage of indomethacin releasedas a function of percentage of drug content and dissolution time was generated by using Eq.3 in order to enable selection of optimum formulation with lactose (Fig.15).

Two multiple regression models obtained in Eq.2 and 3 can be used to estimate the released amount of indomethacin for different values of time at fixed content of drug. It is clear from the results in Table 5 that the drug

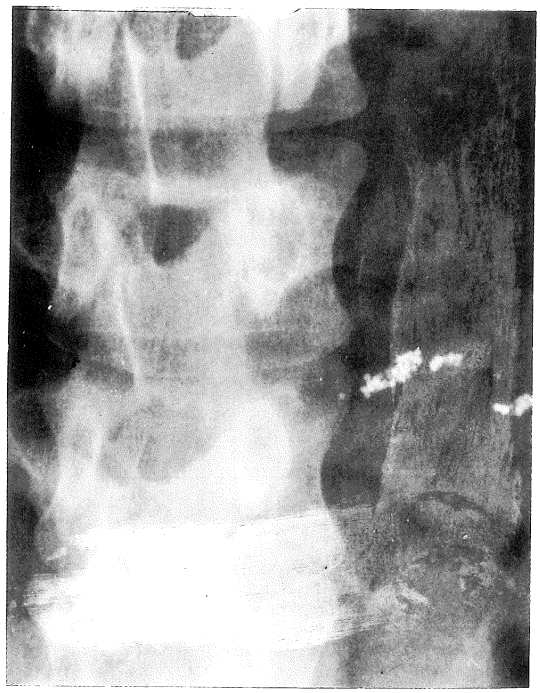

Fig.17-b.X-ray photographs of agar beads containing barium sulfate as a contrast substance in the gastro-intestinal tract(10 min)

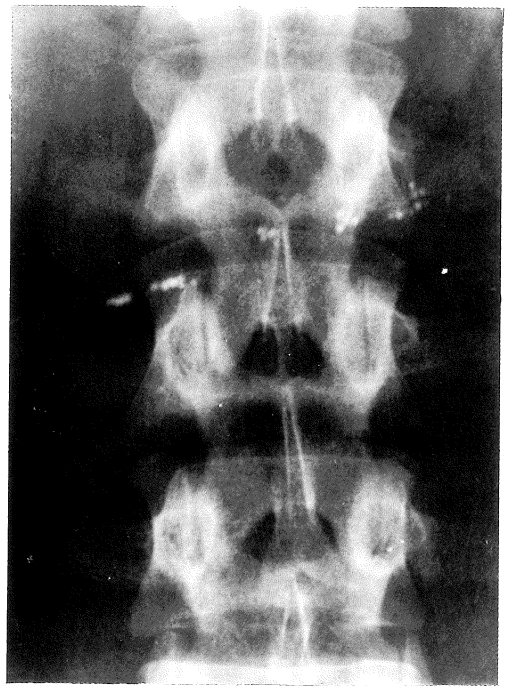

Fig.17-d.X-ray photographs of agar beads containing barium sulfate as a contrast substance in the gastro-intestinal tract (195 min)

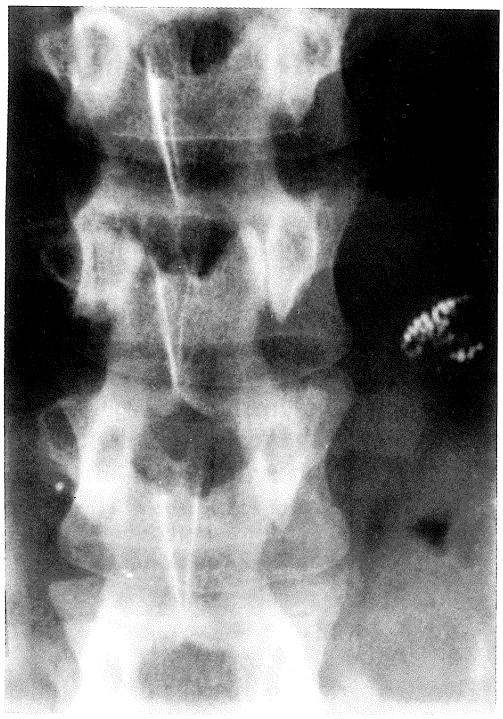

Fig.17-c. X-ray photographs of agar beads containing barium sulfate as a contrast substance in the gastro-intestinal tract (60 min)

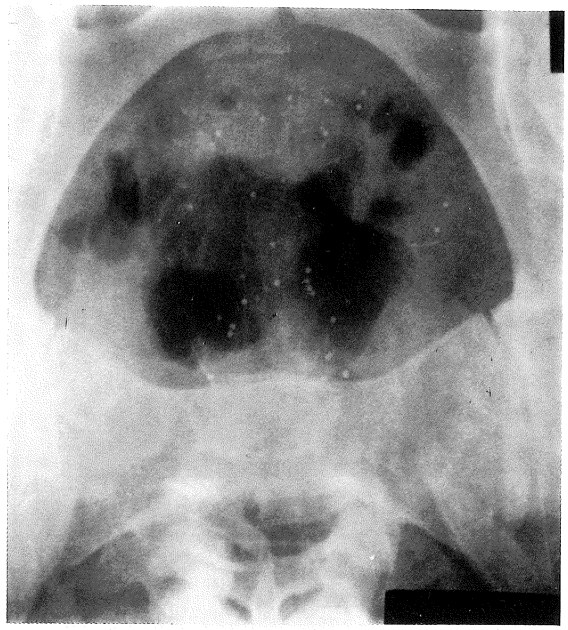

Fig.17-e. X-ray photographs of agar beads containing barium sulfate as a contrast substance in the gastro-intestinal tract (435 min)

release can be increased by adding lactose into the formulations at different ratios. In this case, if Eq.3 is used, in order to find the necessary lactose amount to be added into the formulation a relation between the % indomethacin and lactose amount should be examined. This relationship is found to be approximately linear as given in Fig.16. The equation of this relationship is :

$$y = -2.34x + 105.2 \qquad \text{(Eq.4)}$$

where, x = indomethacin amount %, y=estimated lactose amount %.

Since the indomethacin and agar percent were held constant in the formulation, the amount of lactose to be added into the formulation can be estimated by setting % indomethacin content found by using Eq.3 into the Eq.4.

3- In vivo studies

X-ray photographs taken are shown in Fig.17. On the X-ray examination of the agar beads containing barium sulfate, it was observed that the hard gelatin capsule taken by a healthy volunteer was dissolved in a few minutes and the beads were delivered to the gastric medium. Agar beads have remained in stomach during the first one hour and distributed in small intestines during progressive hours. It is known that the behaviour of a pharmaceutical dosage form in the gastrointestinal tract is important in order to obtain a meaningful relation between in vitro and in vivo drug release results. We also know that a floating dosage form may have a longer gastric emptying time (24-26). In this study, the prepared agar beads were not floating in the dissolution medium due to the fact that they have a higher density than the medium and, thus, they remained in stomach about one hour and then passed towards guts. Indomethacin is a substance which does not dissolve in gastric medium, but dissolves in pH 6.2 phosphate buffer solution. The dissolution studies of indomethacin are carried out in pH 6.2 phosphate buffer solution according to USP XXII. Therefore, the results obtained from dissolution studies which were carried out in dissolution of pH 6.2 can reflect the in vivo performance of indomethacin released from formulation, if the formulation leaves stomach in the shortest possible time.

In summary, the results of the present study clearly demonstrated that agar was useful for the preparation of beads which exhibit sustained release of indomethacin. A possible advantage of the agar beads over conventional sustained release formulations is complete release of the drug from the beads, since some of the commercial sustained release preparations have been reported to fail to release all of the drug in the preparation. Lactose was found to increase the release of indomethacin when added to the formulation. By evaluating the in vitro release results of indomethacin from agar beads prepared with or without lactose mathematically, two equations were estimated. The equations proposed could be used in designing the polymer-drug ratios to be used in the formulation stages of the experiments.

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