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Heating and Chemical Denaturation of Egg Albumin Matrix and its Effect on the Release Kinetics of Theophylline from Tablets

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Abstract: Egg albumin (EA) matrix tablets containing theophylline (TH) were prepared by direct compression using 13 mm die and punch and a hydraulic press (3 ton compressor) after simple dry mixing of these two ingredients. Chemical denaturation of EA using both glutaraldehyde and formaldehyde as cross linking agents showed a remarkable decrease in TH release rate from matrix tablets when compared with untreated and heat denatured TH matrix tablets. The release rate was further reduced when mutual chemical and heating denaturations were applied. In all cases release mechanism was square root of time dependent indicating diffusion type release. It was found that heating for 1 h at only 65°C was enough to produce the release retardation effect, whereas, heating beyond 65°C (up to 105°C) had no significant effect on the release rate. Heat denatured tablets also showed a Higuchian release pattern. The release rate of heat denatured (at 105°C for 1 h) matrices were slightly decreased than the unheated tablets. However the duration of heating (20 to 100 min) had no significant effect on the release rate.

Key words: Sustained release, egg albumin, theophylline, denaturation, release kinetics

INTRODUCTION

The fluctuating drug concentration in tissue following administration of conventional dosage forms lead to an insufficient influence on the mechanisms of diseases and are related to the excessive uses of a drug. Initial over dosing produces a high frequency of side effects, leading under certain circumstances to damage[1,2]. For optimizing drug effects various oral dosage forms have been prepared that control the rate of drug delivery. The simplest forms consist of a drug dispersed in a polymer, the polymer playing the role of a matrix[3,4].

Sustained release (SR) or controlled release (CR) product could provide an initial sufficient amount of drug to initiate a rapid onset of desired therapeutic response and an additional amount of drug that maintain the response at the initial level for a desired period of time beyond the activity resulting from conventional multiple dose. Biodegradable polymers have become increasingly important in the development of controlled release systems. Recently natural polymers such as polysaccharides and proteins have received much attention in the pharmaceutical field owing to their good biocompatibility, biodegradability and mucosal nonsensitivity[5,6].

EA becomes rubby solid when denatured by formaldehyde and glutaraldehyde as chemical cross linking agents[7,8]. The term denaturation has been used to describe the gross alteration of the protein, while there is some disagreement as to the use of the term denaturation, it is best defined as any change in any property of a protein that does not involve the rupture of the basic peptide bonds[9]. In this study, the effect of chemical and heating denaturation of EA on the release characteristics of drug from EA-TH matrix tablet was investigated. Theophylline was used in this study as a marker drug.

MATERIALS AND METHODS

The Egg albumin was purchased as a guaranted raw material from BDH Ltd. UK. Theophylline, magnesium stearate and all other ingredients were of analytical grade. Theophylline BP grade was taken as a marker drug.

Preparation of EA matrix tablets: Sixteen gram EA and 4 g TH were mixed in a container using a mini laboratory drum mixer machine (fabricated in our laboratory) for 15 min. Matrix tablets (250 mg each) were prepared from this blend using a PERKIN-ELMER IR hydraulic compressure machine (U.K.) at 3 ton compression pressure. A 13 mm die-punch toolings were used for compression. The tooling surface was pre-lubricated with a suspension of magnesium stearate in acetone, prior to each tablet compression.

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Denaturation of prepared tablets

Heating method: Heating denaturation of the EA matrices were done by heating the prepared tablets at 65, 85 and 105°C for 1 h. Furthermore, denaturation by heating at 105°C was also done by heating tablets for different time intervals of 20, 50 and 100 min.

Chemical method: A group (40) of EA tablets were taken in a desiccator of 10 L capacity and exposed to gluterladehyde and similarly another group (40) to formaldehyde at 25°C for 36 h. Twenty tablets of these chemically denatured groups were further denatured by heating at 105°C for 1 h.

Dissolution studies: Dissolution tests were carried out for EA matrix tablets using Electrolab Tablet Dissolution Tester U.S.P (XXI) TDT-06 (Bombay, India) with a rotation of 100 rpm at 37°(± 2)°C and 1 L distilled water as dissolution medium. For heat denaturated tablets an aliquot of 5 mL sample solution was withdrawn from each vessel at regular intervals of 5 min during first 20 min, then at every 10 min up to 80 min and thereafter at every 20 min for a total of 220 min. In case of chemical denaturation same method was used except that 20 min interval was continued up to 140 min and thereafter at every 40 min for a total of 260 min, while 40 min time interval was continued for a total period of 380 min in case of tablets denatured by both chemical and heating method. With drawn samples were replaced by equal volumes of distilled water. The samples were filtered and absorbance was measured under a double beam spectrophotometer at λ_{max} 274 nm.

RESULTS AND DISCUSSION

Figure 1 shows the percent release vs. square root of time and revealed almost straight lines. This indicated a Higuchian release pattern that is a diffusion release mechanism of theophylline from EA-TH matrices[10]. Here the drug release follows Higuchian basic equation for matrix tablets[10-11]:

$$Q = [(D \epsilon Cs / \tau)(2A - \epsilon Cs)t]^{1/2}$$

Q is the amount of drug released per unit surface after time t, D is the diffusion coefficient of drug in the medium, τ is the channel tortuosity of the matrix, ϵ is the porosity, Cs is the solubility of the drug in the medium and A is the initial loading dose of the drug. A high tortuosity means that effective average diffusion path way is large and a low porosity means low space available for drug dissolution[11].

Release rates were calculated from the slopes of these lines and plotted against heating temperature (Fig. 2). It indicated that release rate was much slower after heat denaturation than that of unheated EA-TH matrix tablets.

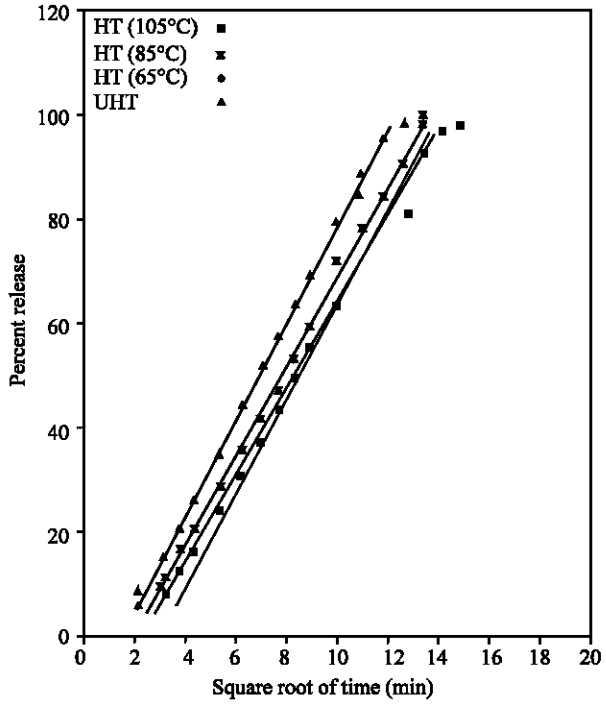

Fig. 1: Effect of heating temperature on the Higuchian release of TH from EA matrix tablets

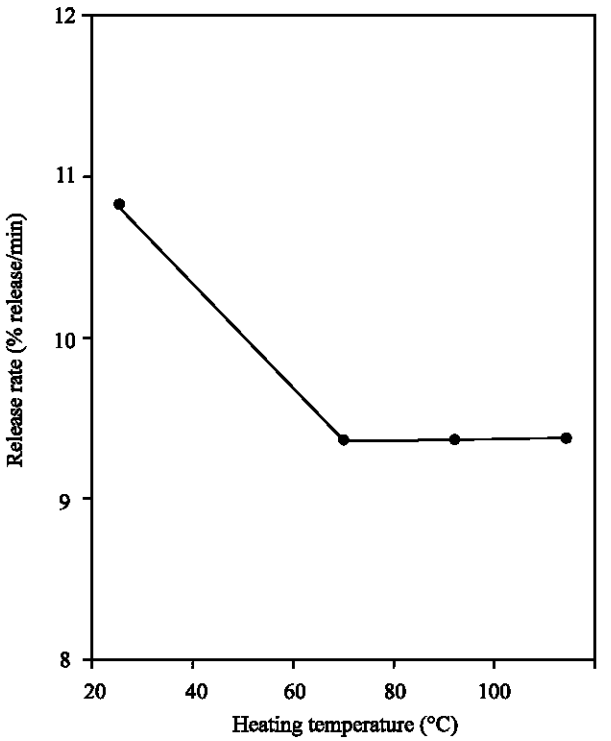

Fig. 2: Effect of heating temperature on the release rate of TH from EA matrices

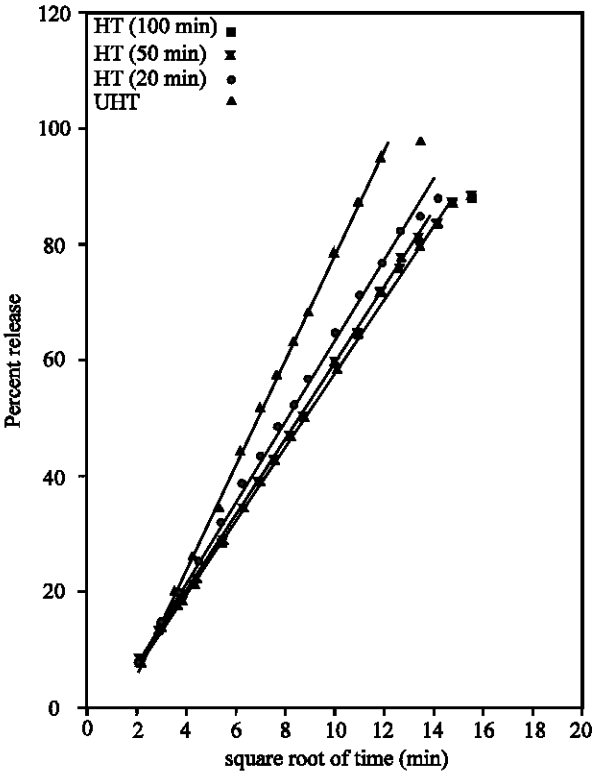

Fig. 3: Effect of heating time on the Higuchian release of TH from EA matrices

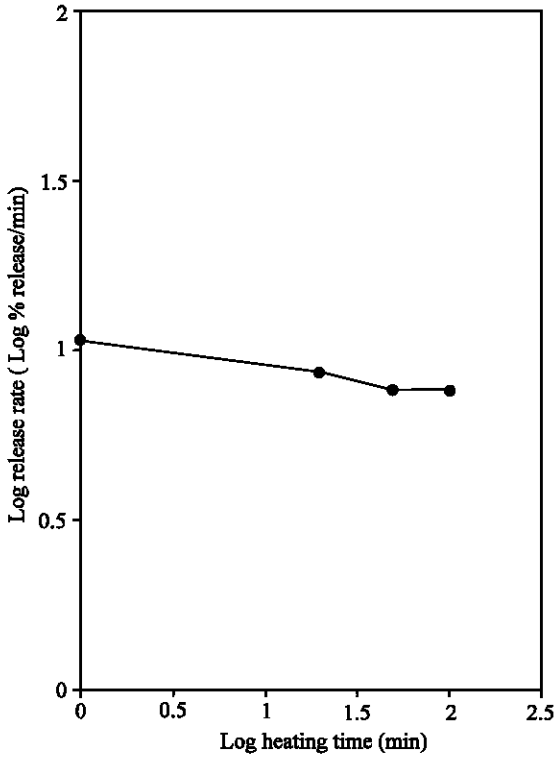

Fig. 4: Effect of heating time on the release rate of TH from EA matrices

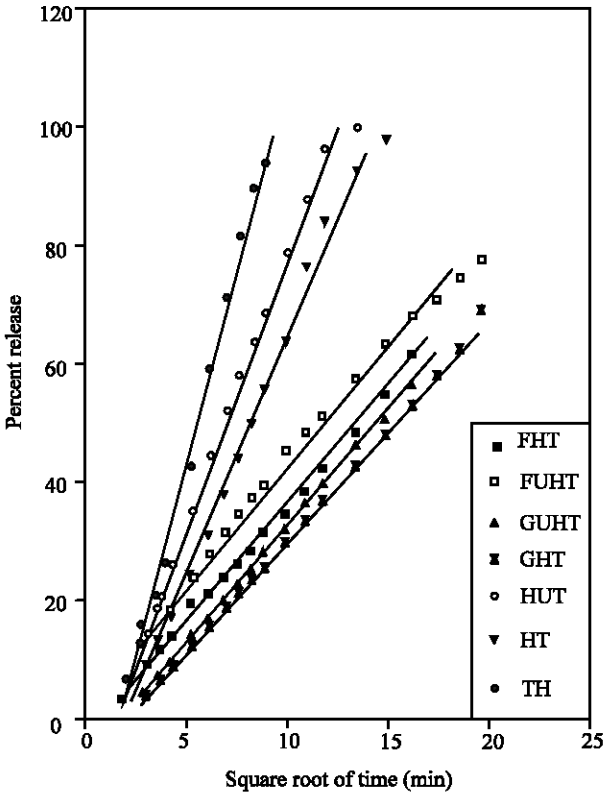

Fig. 5: Effect of formaldehyde and glutaraldehyde cross linking on the Higuchian release of TH from EA matrix tablets. UHT=Unheated, HT=Heated, FUHT=Formaldehyde Unheated, FHT=Formaldehyde Heated, GUHT=Glutaraldehyde Unheated, GHT=Glutaraldehyde heated

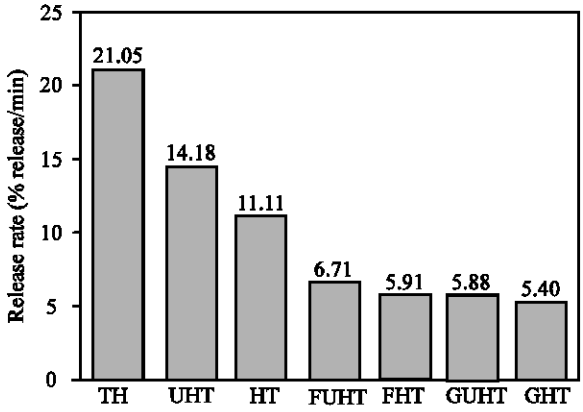

Fig. 6: Effect of formaldehyde and glutaraldehyde cross linking on the release rate of TH from EA matrices. UHT=Unheated, HT=Heated, FUHT=Formaldehyde Unheated, FHT=Formaldehyde Heated, GUHT=Glutaraldehyde Unheated, GHT=Glutaraldehyde heated

It was also clear that further increase in denaturation temperature over 60°C had no significant effect on drug release.

Here, physical method of cross-linking has been done by dehydrothermal treatment i.e. heat denaturation[8]. Heat denaturation has delayed somewhat the *in vitro* dissolution of TH from the EA matrix tablets in comparison to the unheated samples. It is due to the coagulation effect of heating on EA[7,9].

Sugibayashi *et al.*[12,13] reported that an increase in denaturing temperature of EA matrix decreases matrix porosity and increases channel tortuosity in the matrix, leading to decreased drug release. But the release pattern at 65, 85 and 105°C are almost the same. This may be due to the fact that at 60°C, complete denaturation of EA is reached (60°C is coagulation temperature of EA) and therefore, further increase in temperature has no substantial effect on drug release.

Figure 3 shows that release rate of TH from EA matrices was not changed as a function of heating duration. Retardation occurs moderately after heating at 105°C. But prolonged time did not add any significant release hindrance. Figure 4 shows that there is a linear correlation between log release rate and log heating time. It shows that the log release rate is slightly decreased with increasing log heating time.

Figure 5 showed the effect of chemical denaturation of egg albumin using formaldehyde and glutaraldehyde on drug release. The obtained straight lines relation for percentage of drug release and square root of time indicated fitness with Higuchi model at this condition. The calculated slope values revealed that release retardation was possible by chemical denaturation (Fig. 6).

It could be noticed that drug release has been significantly retarded by the chemical denaturation. At 60 min, the drug release from untreated EA matrix tablets was above 80% whereas in case of glutaraldehyde and formaldehyde this was only 20 and 22%, respectively, which is about four times retardation.

Chemical denaturation with aldehyde takes place by chasing the formation of methylene bonds to amino groups of albumin according to the following equation[14]:

$$R\text{-}NH_2 + HCHO + R\text{-}NH_2 \rightarrow R\text{-}NH\text{-}CH_2\text{-}NH\text{-}R + H_2O$$

The three dimensional tight net work formed by interaction covalently cross linking[8] may retard the release of TH markedly from matrices.

EA-TH matrix tablets were prepared with an aim to study the effect of chemical and heating denaturation of EA on the release of TH from these EA-TH matrices. Chemical denaturation with heating showed a remarkable decrease (almost 4 times retardation) in TH release rate from matrix tablets and only heating denaturation on the other hand produced a little retardation. Throughout the study, the release pattern was a diffusion controlled one i.e. Higuchi Matrix Dissolution system.

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