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PREPARATION AND EVALUATION OF GELATIN-BASED DISC MATRICES FOR THE DELIVERY OF ISONIAZID

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Abstract
The objective of the present study was to formulate disc matrices based on type B gelatin and to evaluate their potential for use as a vehicle for the delivery of isoniazid. Gelatin hydrogel was prepared in an aqueous solution and subsequently cross-linked with glutaraldehyde. The cross-linked hydrogel was cut into discs and dried under vacuum in a dessicator to form the disc-shaped matrices which were further evaluated for swelling, drug loading and *in vitro* drug dissolution properties. The effect of varying the duration of cross-linking time on these evaluated properties was similarly evaluated. The results obtained showed that the matrices exhibited significant swelling in simulated gastric fluid (SGF) without pepsin (pH 1.2), simulated intestinal fluid (SIF) without pancreatin (pH 6.8) as well as in water. Increase in cross-linking time resulted in increased swelling of the disc matrices in the different swelling media studied. Loading efficiency was similarly observed to increase with increase in cross-linking time with values ranging from 60 to 95 %. Release of isoniazid from the matrices was higher and more rapid in SIF than in SGF and was sustained for more than 24 h. Drug release from the formulated matrices was found to obey the Higuchi model release mechanism. This study, therefore, suggests that disc matrices based on gelatin hold some potential as a vehicle for the controlled delivery of isoniazid.

Keywords: isoniazid, disc matrices, gelatine, hydrogel, cross-linking

INTRODUCTION

Controlled drug delivery occurs when a polymer, whether natural or synthetic is judiciously combined with a drug or other active agent in such a way that the active agent is released from the material in a predesigned manner. The release of the active agent may be constant over a long period, it may be cyclic over a long period or it may be triggered by the environment or other external events (Muller, 1987). The controlled delivery of drugs is important for a broad range of pharmaceutical formulation and offers numerous advantages compared to the conventional dosage forms which includes: improved efficacy, increase in the therapeutic index of the drug and reduction of toxic side effects, optimization of drug action by adjustment of the drug release rate or change in the rate of drug deposition, the design of pro-drugs and the administration of the drug at its desired site of action (drug target). All these improve patient compliance and convenience (Muller, 1987).

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Controlled drug delivery by controlled release formulation has been used for various routes of administration including the oral (Davies, 1987) rectal, buccal, nasal and ocular delivery systems (Hermia *et al.*, 1986; Robinson, 1986; Fitzgerald *et al.*, 1987) and a large number of systems for parenteral applications (Bundgard *et al.*, 1982). In addition to controlled drug delivery, targeting the drugs to diseased or injured areas is one of the most important aspects in drug delivery. Systems used for site-directed (targeted) delivery include macromolecular prodrugs (Hoes *et al.*, 1985), antibody-drug conjugates (Burstein, 1976; Hurwitz, 1982), drugs bound to macromolecular carriers, particulate systems such as liposomes, and a range of solid microspheres and nanoparticles. Suitable drug carriers such as polymers (sufficient as barrier to drug movement) ensure that a sufficient drug dose gets to the diseased area. Recent research on polymers holds out hope of obtaining new polymers for drug delivery devices essential for the efficient use of today's potent and toxic drugs.

Hydrogel consists of a network of polymer chains that are water insoluble, sometimes found as a colloidal gel in which water is the dispersion medium (Wikipedia, 2011) Hydrogels are highly absorbent polymers which can absorb a significant amount of water (>20 % of their mass) without dissolving or losing their integrity. Hydrogels e.g. polyvinyl alcohol can contain over 99 % water and they can be natural or synthetic polymers (Pal *et al.,* 2007). Hydrogels also possess a degree of flexibility very similar to natural tissue, due to their significant water content, soft and rubbery consistency (which minimizes mechanical irritation to surrounding tissue) and low interfacial tension with water or biological fluids (minimizes protein adsorption and cell adhesion) and therefore are biocompatible in nature and non-irritating to soft tissue. Their ability to absorb water is due to the presence of hydrophilic groups such as -OH, -CONH, -$CONH_2$, -COOH and -SO_3.

Hydrogels have been used for various biomedical applications which include the following: as scaffolds in tissue engineering and regeneration, as implants, as biosensors, in disposable diapers and incontinence products, as contact lenses, artificial corneas and catheters, as medical electrodes, as water gel explosives, as reservoirs in topical drug delivery particularly ionic drugs, delivered by iontophoresis, and as vehicles in ointments for treating topical fungal and viral infections and periodontal diseases (Kudela, 1985). Hydrogels are classified into pH sensitive, thermosensitive, electrically sensitive hydrogels and enzyme sensitive hydrogels based on their sensitivity to different stimuli (Kudela, 1985). In polymer science, the use of cross-links to promote a difference in a polymer's physical properties is referred to as cross-linking. When polymer chains are linked together by cross-links, they lose some of their ability to move as individual polymer chains. For example, a liquid polymer, which possesses freely flowing chains, can be turned into a "solid" or "gel" by cross-linking the chains together. Cross-linking inhibits close packing of the polymer chains, preventing the formation of crystalline regions. The restricted molecular mobility of a cross-linked structure limits the extension of the polymer material under loading. This means that when a polymer is stretched, the cross-links prevent the individual chains from sliding past each other. In the process, the chains may straighten out, but once the stress is removed they return to their original position and the object returns to its original shape. It is a well

known fact that polymers with a high enough degree of cross-linking have "memory". With this "memory", such cross-linked polymers can be exploited for a number of useful purposes including modifications for improved drug delivery and release.

Several reports have indicated that the cross-linking density, molecular weight, electrical charge of polymers and other factors might have a profound effect on the release rate of drugs from polymer-based multiparticulate drug delivery systems (Adhirajan *et al.*, 2007). Among these factors, the modification of the cross-linking density is expected to be the most useful for optimizing the release rate of drugs, including peptides, from such systems. Alteration of the cross-linking conditions almost always results in changes in the cross-linking density of biopolymers. Both the prolongation of the cross-linking reaction time and an increase in concentrations of cross-linking agent have been reported to increase the cross-linking density of gelatin and other biopolymers. These reports suggest that polymers with desirable cross-linking density could be obtained by optimizing the conditions of the cross-linking reaction. The cross-linking density of polymers such as alginate and collagen could also be altered by modifying the cross-linking reaction time and the concentration of the cross-linking agent. A previous report has demonstrated that as the cross-linking density became higher, the amount of insulin released from gelatin microspheres in the initial phase decreased (Adhirajan *et al.*, 2007).

Gelatin is a natural polymer obtained by alkaline or acidic pre-treatment and thermal
denaturation of collagen, the most widespread protein in the body. It is biodegradable, biocompatible and non-immunogenic, which makes it suitable for biomedical applications, such as a sealant for vascular prosthesis (Jonas *et al*., 1988) and in drug delivery as hard and soft capsules, hydrogels (Tabata *et al*., 1994) or microspheres (Narayani and Rao, 1994) and in a wide variety of wound dressings (Ulubayram, 2002). Isoniazid is one of the mainstay drugs in the treatment of tuberculosis and is known to be associated with a myriad of adverse reactions which may be dose-related. In addition, isoniazid is known to have a half-life of 1 to 3 hours necessitating at least a twice daily administration. To avoid or reduce toxicity especially in patients with slow clearance of the drug via acetylation, controlled delivery of isoniazid using specialized carriers such as gelatin-based disc matrices may offer some advantages. The objective of this study, therefore, was to develop a carrier system based on cross-linked gelatin disc matrices for the controlled delivery of isoniazid, a known antitubercular drug and to investigate the effect of cross-linking time on the morphology, swelling behaviour and release properties of the formulated matrices.

MATERIALS AND METHODS

Glutaraldehyde (25 % v/v solution), glycine, sodium chloride, monobasic potassium phosphate, sodium hydroxide (BDH Chemicals Ltd Poole, England), type B gelatin (Sigma, Germany). All other reagents were analytical grade and were used as procured from their sources. Distilled water was collected from an all glass still.

Preparation of gelatin disc matrices.

A quantity (5 g) of gelatin was weighed out and transferred to a beaker containing 50 ml of distilled water and stirred (400 rpm) with the aid of a magnetic stirrer-hot plate assembly set at 50 ^{o}C to give a 10 %

w/v solution of gelatin. Then 1 ml of a 25 % solution of glutaraldehyde as cross-linking agent was added and stirring continued until a gel was formed. Cross-linking of the formed gel was continued for the next 1 h followed by addition of 5 ml of 0.01M solution of glycine and equilibration at 37^{o}C for 1 h in a thermostated water-bath to quench the cross-linking action of glutaraldehyde. Discs of about 10 mm diameter by 5 mm thickness were cut out and dried in a desiccator at room temperature over a period of 72 h and the dried matrices were then stored in amber coloured glass bottles in a desiccator. The above procedure was repeated with cross-linking times extended to 2, 3, 4, 5, 6, 12, and 24 h.

Evaluation of the surface characteristics of the disc matrices

The morphological characteristics of the matrices were determined using a optical microscope (Olympus, Japan) equipped with a digital camera. Each sample was mounted on glass slide and viewed in the microscope with a total magnification of ×40. The images were captured and further examined.

Swelling studies

The dried disc matrix was weighed and placed in SGF to swell and at intervals of 10 min for the first 60 min and thereafter at 30 min intervals, the disc was removed from the swelling medium, blotted dry and weighed. The procedure was repeated for the different batches using SIF or distilled water as swelling medium. The swelling index was calculated using this formula:

$$Swelling\ Index = \frac{W2 - W1}{W1}$$

where, $W1$ is the initial weight of the disc matrix, and $W2$ is the final weight after swelling.

Drug loading

A solution of 10 % w/v isoniazid was prepared and 5 ml of this solution was placed in a test tube and the dried disc matrix from each batch was allowed to swell and equilibrate in the test tube for 24 h at room temperature in order to allow sufficient time for maximal swelling and saturation of the swollen matrix with the drug solution. After 24 h the disc matrix was brought out of the drug solution, dabbed with filter paper and dried in a desiccator.

Determination of loading efficiency of the disc matrices

The dry loaded disc matrix was weighed and then soaked in 5 ml of SGF in a test tube. This was allowed to stand for 24 h after which the swollen disc matrix was vortexed. The particles were filtered off and the absorbance of the filtrate, after appropriate dilutions was measured at a wavelength of 292 nm using a UV-Vis spectrophotometer (Model 305, Jenway, England). This procedure was repeated for all the batches of the formulated disc matrices.

***In vitro* drug release study**

The USP basket apparatus was used to study the *in vitro* drug release from the disc matrices. The release behaviour of the drug from different batches of the matrices was studied in SIF, pH 7.4 and in SGF, pH 1.2. A rotating basket set to sink conditions and rotating at 100 rpm was used to generate drug release profiles. Each drug-loaded disc matrix was enclosed in a dialysis membrane (9 cm long), placed in the basket and then lowered into the dissolution medium that was

maintained at 37± 0.5˚C. At predetermined time intervals of 0.5, 1, 2, 3, 4, 5, 6, 7, 8 then 12 h, 1 ml aliquots of the release medium were withdrawn, appropriately diluted and assayed at an absorption maxima of 292 nm, using a Uv-Vis spectrophotometer. At every interval, 1 ml of fresh release medium was added to replace the withdrawn sample.

RESULTS AND DISCUSSION

Figure 1 (A to F) shows the photomicrographs of the different batches of the disc matrices. The internal matrix structure could not be seen but the disc matrices showed different surface characteristics that varied with the cross-linking time. The gelatin disc matrices were observed to become hard after drying for several days in the desiccator. All the formulated batches had a brownish-amber colour after drying. Figures 2 - 4 show the swelling profiles of the disc matrices in distilled water, SGF and SIF. In distilled water, the least degree of swelling was recorded for matrices cross-linked for 1 h while the highest degree of swelling was observed in the batch cross-linked for 5 h. In SGF and SIF, however, water sorption was least in the batch cross-linked for 2 h and highest in the batch cross-linked for 24 h. Generally, water sorption increased with increase in cross-linking time.

The cross-linking activity of glutaraldehyde increased with time leading to stronger bonding and network formation which improved the capacity of the gel to absorb more fluid. An important consequence of prolonged swelling by hydrogels is an increase in the diffusion pathway which can result in decreasing drug-concentration gradient and, thus, a decrease in drug release rate [12]. On the whole, water sorption was observed to vary with the swelling media and followed the pattern: water sorption in SGF ~ SIF > distilled water.

It is discernible from Table 1 that the amount of isoniazid entrapped within the matices increased as the cross-linking time increased. Loading efficiency was observed to be generally high for all batches of the disc matrices and ranged between 59.6 and 94.6 %. Swelling of the disc matrices in an aqueous environment containing the drug and adsorption of the drug into the core (matrix) and on the surface of the formulated matrices were observed to be the principal mechanisms of loading of isoniazid into the matrices. The drug entrapment behaviour of the matrices was similarly observed to bear a correlation to their water sorption behaviour. As noted earlier, it is probable that stronger bonding and network formation which improved the water sorption capacity of the matrices also led to enhanced drug absorption from the aqueous medium in which drug loading took place.

The release profile of isoniazid from the disc matrices in an acidic medium (SGF) and in a physiological pH (SIF, pH 7.4) is shown in Figures 5 and 6 respectively. In both release media, a somewhat biphasic pattern of drug release was observed. This was characterized by an initial drug release which occurred rapidly in less than 30 min into the release experiment in which more than 10 % of the loaded drug was released. This is known as “burst release”. This initial release was followed by a more gradual and

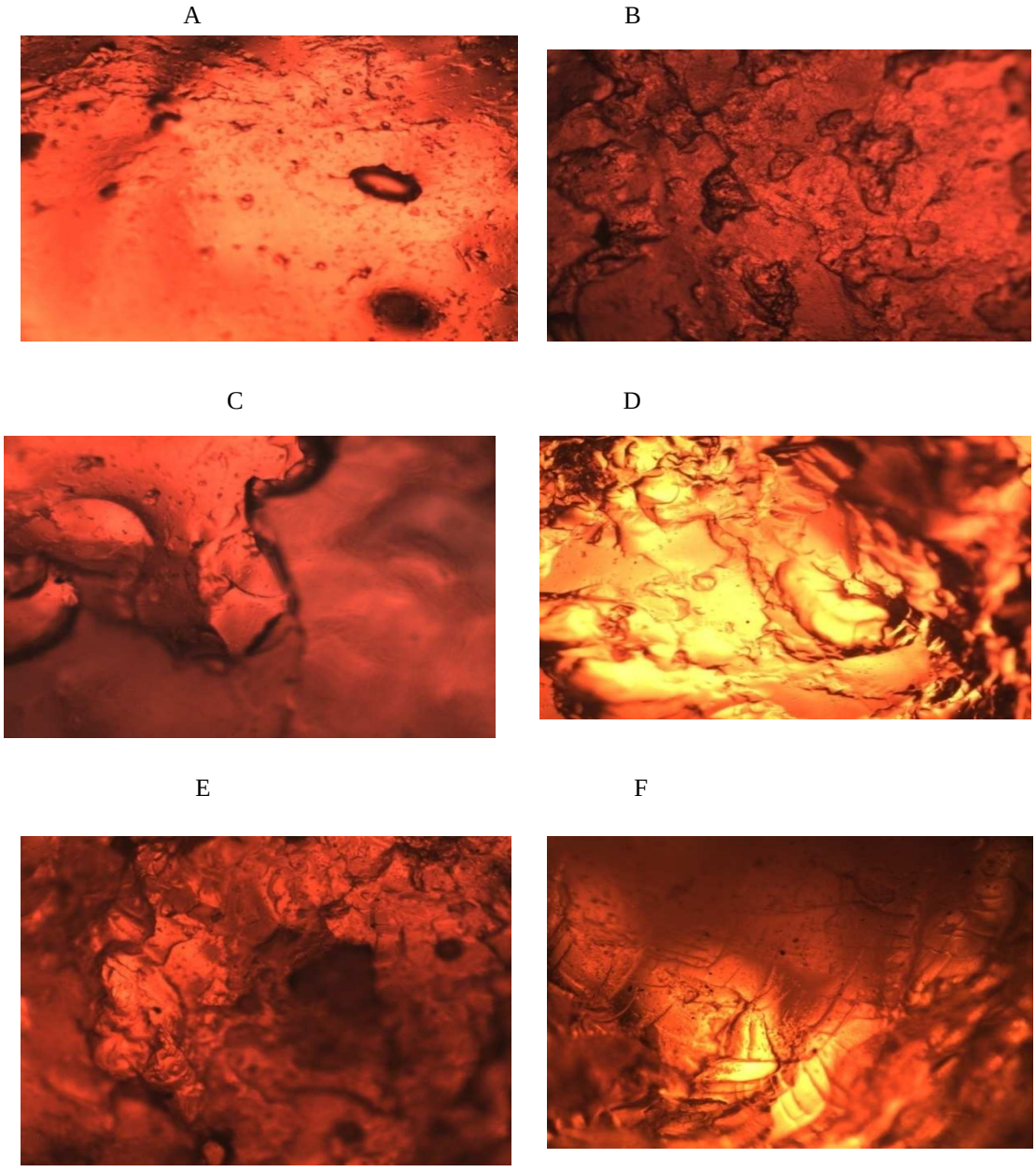

Fig. 1: Photomicrograph of the gelatin disc matrices cross-linked for 1 h (A), 2h (B), 3 h (C), 4 h (D), 5 h (E), 6 h (F)

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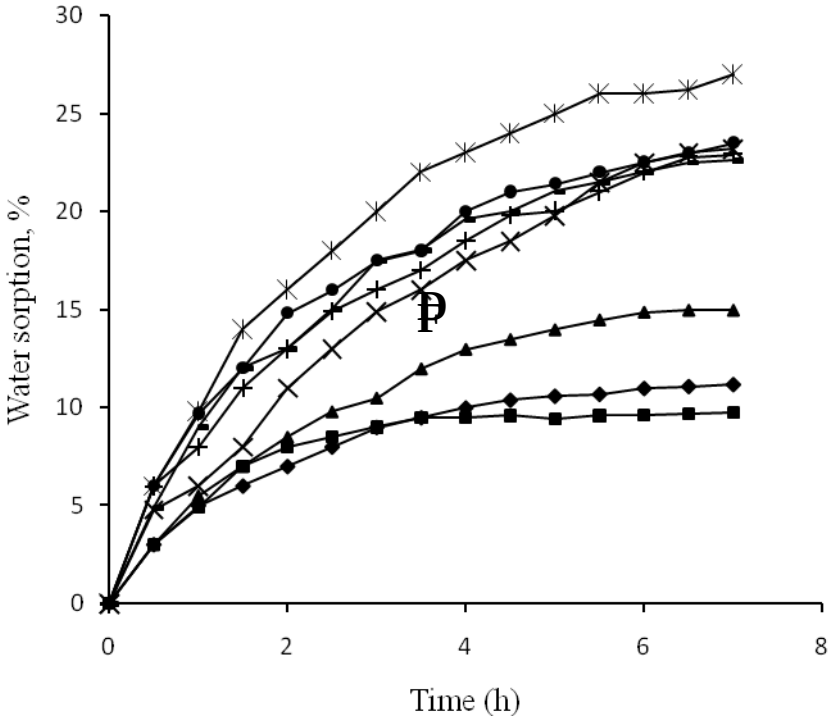

Fig 2: Swelling profile of the disc matrix in distilled water.
—♦— 1 h, —■— 2 h, —▲— 3 h, —×— 4 h, —*— 5 h,
—●— 6 h, —+— 12 h, —▬— 24 h

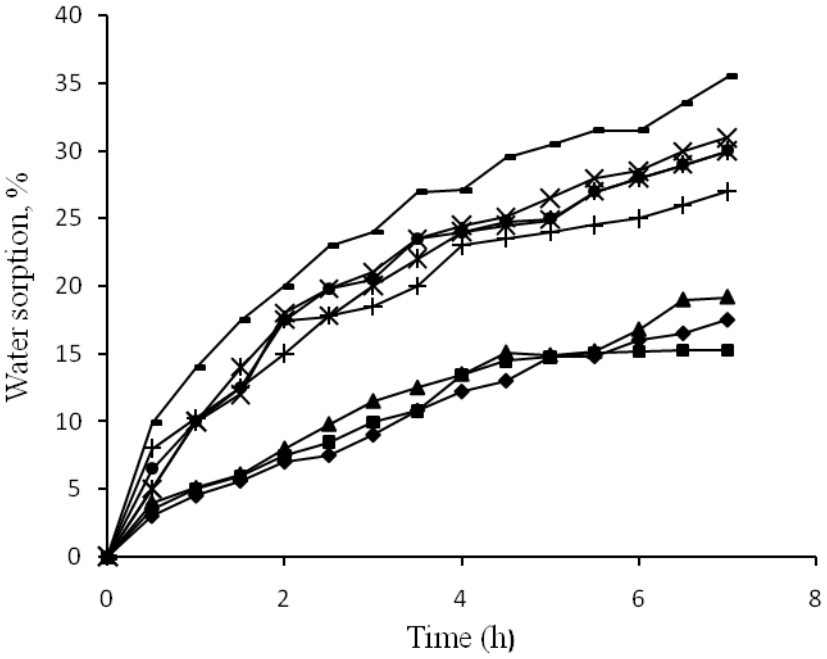

Fig 3: Swelling profile of the disc matrix in SIF.
—♦— 1 h, —■— 2 h, —▲— 3 h, —×— 4 h,
—*— 5 h, —●— 6 h, —+— 12 h, —▬— 24 h

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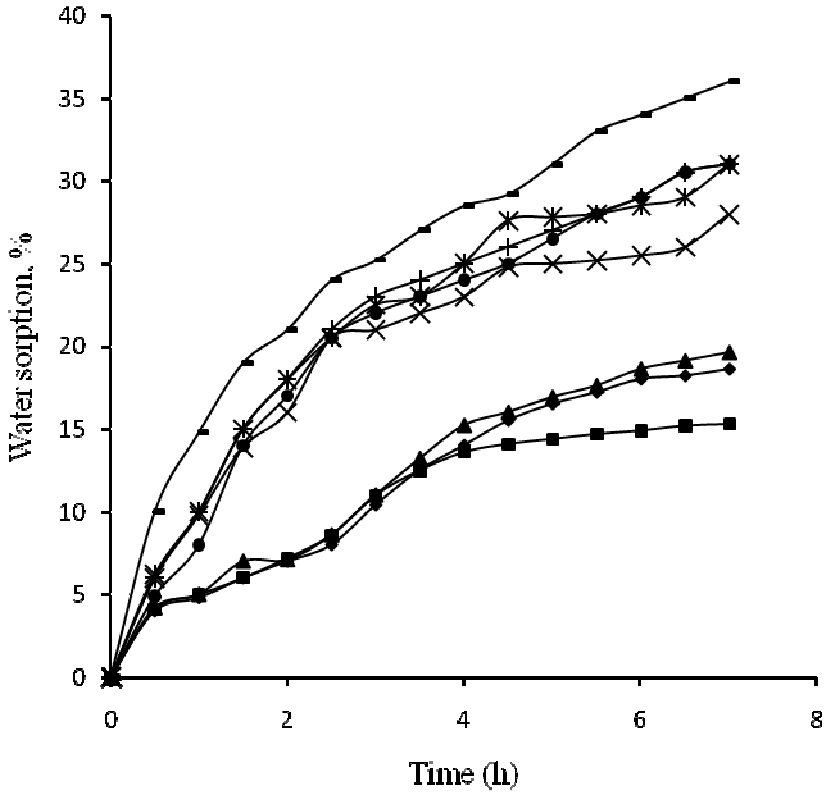

Fig. 4: Swelling profile of the disc matrices in SGF.
—♦— 1 h, —■— 2 h, —▲— 3 h, —×— 4 h, —*— 5 h —●— 6 h, —+— 12 h, —▬— 24 h

Table 1: Effect of different cross-linking times on the loading efficiency of the disc matrices

Cross linking time (h)	Loading Efficiency, %
1	59.6
2	69.6
3	70.4
4	81.3
5	83.2
6	88.4
12	91.6
24	94.6

extended release over 12 h. The amounts of isoniazid released as a result of burst effect may likely represent the amounts that adhered weakly to the surface of the formulated matrices. The remaining amounts which were released in a more gradual pattern most likely represented the amounts that were entrapped into the core (matrix) of the disc matrices. Over the 12 h release period, higher amounts of isoniazid were released in SIF than in SGF. In SGF, not more than 65 % and not less than 45 % of the loaded drug was released over the whole period whereas in SIF, between 60 % and 75 % of the loaded drug was released over the same period for all batches of the formulated disc matrices. The release experiment was carried out for 12 h with sampling at hourly intervals. Even after 12 h, drug release from the matrices had not gone to completion. In order to determine the mechanism of drug release from the formulated disc matrices, the release data were fitted into Higuchi diffusion model of drug release by plotting the amount of drug released in percent against the square root of time. Correlation coefficients in the range of 0.967 to 0.985 were obtained indicating that release of isoniazid from the formulation followed the Higuchi diffusion model. Drug release from most polymer-based matrices is known to occur through a combination of one or more mechanisms including but not limited to desorption, diffusion and matrix erosion and/or disintegration. In the present study, the likely predominant mechanisms of drug release based on the results of the swelling and in vitro dissolution studies would be expected to be one or more of desorption, diffusion and disc erosion and/or disintegration.

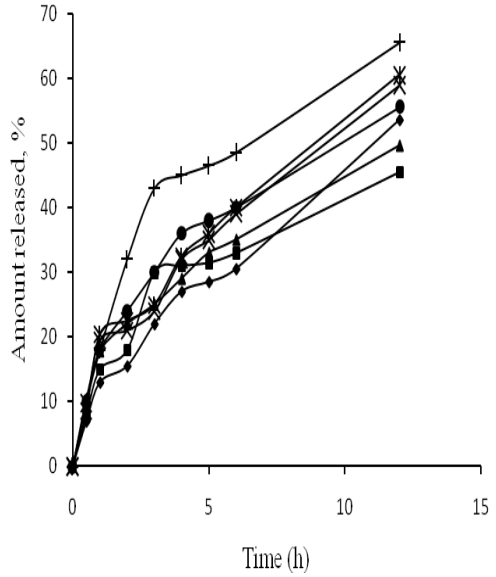

Fig. 5*:* Release profile of isoniazid from the disc matrices in SGF.
—♦— 1 h, —■— 2 h, —▲— 3 h, —×— 4 h, —*— 5 h, —●— 6 h, —+— 12 h

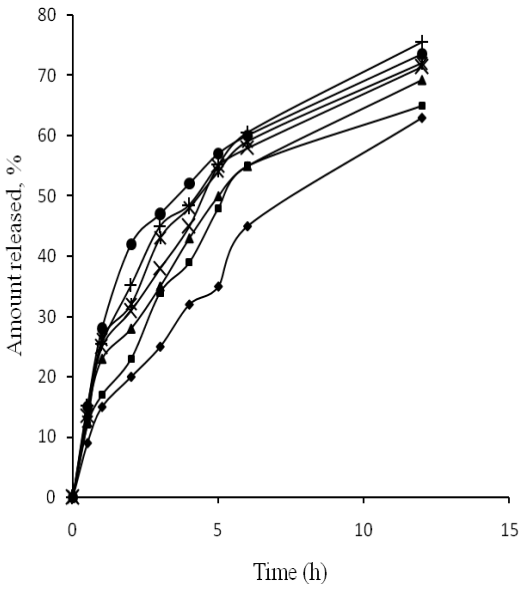

Fig.6: Release profile of isoniazid from the disc matrices in SIF.
—♦— 1 h, —■— 2 h, —▲— 3 h, —×— 4 h, —*— 5 h, —●— 6 h, —+— 12 h

CONCLUSION

Disc matrices based on gelatin exhibited marked water sorption capacity and entrapped substantial amounts of the loaded drug depending on the time for the cross-linking reaction. Release of the loaded drug was sustained for well over 12 h and appeared to have taken place in a controlled manner. This study, therefore, suggests that disc matrices based on gelatin hold some potential as a vehicle for the controlled delivery of isoniazid.

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