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OPTIMIZATION OF CONTROLLED DRUG RELEASE THROUGH MICROPELLETIZATION

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ABSTRACT

Micropelletization technique using crosslinked gelatin matrix was chosen to evaluate its utility in controlled release medications. Trimethoprim, which has a very high solubility gradient in gastric pH, was selected in this investigation. Micropellets were formed by the modified spray congealing technique. The effects of exposure of the crosslinking agents to the gelatin matrix of the micropellets on the effectivity as the controlled release drug delivery system were investigated. The total product yield, content uniformity and the reproducibilty of the sucessive batches were decidedly superior to either the pure drug or the non-crosslinked ones. Particle size distribution was observed to vary depending on the content of the crosslinked gelatin in the micropellets. Scanning electron micrographs confirmed the porous surface topography of the micropellets. The drug release characteristics was suggested as the diffusion controlled first order dissolution process.

INTRODUCTION

The consideration of the effects of the gastric emptying time becomes a very important factor in the design of controlled release oral dosage forms. The size of the dosage form considerably affects the gastric emptying time for the particular dosage form[1]. Single unit preparations tend to follow the food which has the small intestinal transit time between 3 and 8 hours[2]. When the unit of the dosage forms are less than 1.5 mm, they can easily pass the pyloric region of the intestine even when the sphincter is closed. This phenomenon avoids the dependency of the dosage form on the individual's intestinal transit time[3], which widely varies on the emotional state and the diet[4].Comparing the controlled release multiple units with the single units, Bechgaard et al[5] specifically stressed that the multiple unit dosage forms, comprising of hundreds of mini-subunits, present a highly preferable alternative, which is due to the greater predictability and reproducibility of its therapeutic effects and will have a much lowered risk of dose dumping, side effects and gastrointestinal irritations. Controlled release dosage forms such as the solid spherical micropelleted dosage forms are becoming very popular in therapeutics as attempts are being made to extend the plasma half life of certain drugs. Generally, micropellets are spherical bodies formed from a mass of finely divided particles by a continuous rolling or tumbling motion[6]. Micropellets ranging from 0.4 to 1.5 mm are the most important in galenical pharmacy.

One of the most important method for the preparation of pellets, of different size for various uses, is the spray congealing technique. The embedding of drugs into spherical

matrices by the spray congealing method was reported previously [7, 8]. The present investigation involves the design of micropellets of crosslinked gelatin matrix and the study of the drug release profile from the dosage form in order to produce an optimum controlled release properties. Trimethorprim, with a biological half life of 8.9 ± 0.9 hours[9] and very high solubility gradient in gastric pH, was selected as the core material for fabrication of the micropellets.

EXPERIMENTAL

Materials:

Trimethoprim I.P. was supplied by the courtesy of Smith Stanistreet Pharmaceuticals Limited, Calcutta. Gelatin (Type 1, 300 Bloom, Sigma Chemical Company, St. Louis, U S A), Liquid Paraffin and Light Liquid Paraffin - I.P., Glutaraldehyde A.R. 25% w/v (E. Merck, India), Formalin A.R. 35% (E. Merck, India) were obtained commercially and used as received. Trimethoprim was sieved to yield particles in the range of 250 µm.

Preparation of the Dosage Form:

The formulations were prepared in the proportions of 3: 10, 1: 2 and 1: 1 as the drug to gelatin ratio. Sufficient amount of drug was slowly incorporated in 30% w/v of gelatin sol in water. The homogeneous drug gelatin mixture was poured in a constant and steady stream into warm, 55 - 60°C, 400 gms of mixture of liquid paraffin at the absolute viscosity of 23.92 cP at 55°C, achieved by blending 40% v/v liquid paraffin I.P. and 60% v/v light liquid paraffin I.P. The system was stirred uniformly at 300 - 350 r.p.m with a paddle stirrer.

After stirring for about 15 minutes, the content of the vessel was quickly cooled down to about 5 - 10°C by placing ice mixture around the beaker. This temperature was maintained till the gelatin microdrops formed perfect gel. The system was further stirred for about 15 minutes and 30 ml of chilled isopropanol was added at the rate of 3 ml/min. Then the beaker containing gelled micropellets were kept in a refrigerator overnight to allow the completion of the gelling process.

After overnight freezing, the micropellets were filtered through a # 100 mesh nylon cloth and washed several times with chilled ethyl ether to remove the traces of liquid paraffin. Then they were dried in a vacuum desiccator for 24 hours to a moisture content of about 5 - 10%.

Crosslinking of the Gelatin Matrix :

The crosslinking of the gelatin matrix was achieved by three techniques, which are described below :

i) Crosslinking by glutaraldehyde/formaldehyde-isopropanol mixture : Each size range of the micropellets were dipped into 10% w/w solution of glutaraldehyde/formaldehyde in isopropanol in a covered glass vessel, then in a refrigerator at 2 - 5°C for 24 hours and dried in air.

ii) Crosslinking by glutaraldehyde/formaldehyde vapour: Each size range of micropellets were placed over a screen of # 100 mesh kept inside a desiccator previously saturated with the vapour by transfering 50 ml of 35% w/w of formaldehyde or 25% w/w glutaraldehyde. The micropellets were exposed to the vapour for crosslinking of the gelatin for variable times. Time to time, the micropellets were moved to have uniform

crosslinking. Then the micropellets were kept in air for 24 hours at ambient temperature and then dried at 35°C for complete removal of adsorbed glutaraldehyde/formaldehyde By varying the duration of crosslinking, it was thoughtto control the drug release.

iii) *In situ* crosslinking : One hundred fifty ml solution of glutaraldehyde/formaldehyde was added to the system after the gelatin microdrops formed perfect gel, instead of water abstracting chilled isoprapanol. Then the total mixture was kept in refrigerator for overnight to allow complete crosslinking.

Evaluation of Micropellets

Total yield :

The micropellets were weighed accurately on keeping in the vacuum desiccator until constant weight was attained.

Scanning electron microscopy :

The micropellets were fixed on the aluminium mounts and were coated with gold using a sputter coater device. The photograph was taken on HITACHI S - 415 A Scanning Electron Microscope.

Content Uniformity

An accurately weighed 100 mg of the powdered micropellets was transfered into a 250 ml glass stoppered Erlenmeyer flask, using 50 ml of 20% w/w of acetic acid solution in water. The

content of the flask was left to stand for 18 hours at ambient temperature with constant shaking. The liquid was filtered and the aliquot was assayed spectrophotometrically[10].

Particle size analysis :

Particle size distribution was determined using standard sieve shaker, having double gyratory and vibratory movements.

In vitro dissolution :

The *in vitro* dissolution study was undertaken in U S P XX dissolution apparatus with the basket covered with # 100 mesh nylon screen bonded permanently to the inner wall. Dissolution studies were carried out at the designated pH profile, which was reported to simulate the gastro-intestinal pH conditions[11]. The aliquots of dissolution were assayed spectrophotometrically[10].

RESULTS AND DISCUSSIONS

The formation of the micropellets is a physical phenomenon. When the drug is dispersed in the gelatin sol it acquires an enormously large surface area. When this gelatin sol is poured in a hydrophobic medium, as liquid paraffin, while stirring, the strong cohesive forces among the molecules of gelatin sol, under the influence of the hydrophobic nature of the surrounding medium, converts itself into droplets of different sizes. Due to the natural tendency of any matter to achieve the minimum surface area, the droplets achieve spherical shape. Three factors are associated with this phenomenon ;

- the cohesive force of the gelatin sol,
- the hydrophobic nature of the surrounding medium,
- continuous rolling or tumbling motion of the soft gelatin microdrops.

Factors Affecting Micropelletization:

The following factors were observed to be very important in order to achieve good physical properties of the micropellets:

Viscosity of the paraffin medium:

Experiments were undertaken using the media of different viscosities achieved by blending light liquid paraffin and liquid paraffin in the volume proportion of 1:3, 3:2 and 1:1. It was observed that optimum viscosity at the temperature of the manufacture played an important factor on the physical characteristics of the micropellets. Increasing the viscosity produced higher percentage of unacceptably large micropellets. The optimum viscosity of 23.92 cP at 55^{o}C was observed to be best in obtaining good particle size distribution,

Viscosity of the drug - gelatin mixture :

The viscosity of the drug gelatin mixture at the particular temperature influenced the diameter of the stream of its addition. This viscosity is again influenced by the total amount of the drug dispersed into the gelatin sol and its water content. At higher viscosity of the drug - gelatin mix-

ture, much larger micropellets were formed, whereas, at lower viscosity much smaller micropellets were formed while keeping the temperature unchanged.

Degree of agitation or stirring speed :

The stirring speed was found to be one of the most important factors contributing to the size distribution of the micropellets. The percentage of small micropellets increased when increasing the stirrer speed. At the same time, the percentage of large micropellets increased on decreasing the stirrer speed. Therefore, an optimum stirrer speed is always desirable, which was found to be in the range of 300 - 350 r.p.m.

Temperature :

Temperature change of the medium had critical influence over the size of the micropellets. Finer micropellets were obtained if there was a delay in lowering of the temperature of the medium during the soldification. The bigger micropellets initially formed were broken down into finer ones due to softness. The high temperature of the medium ($>70^{o}C$) at the beginning of the process made the gelatin droplets to reduce further their sizes which was again influenced by the speed of the stirrer. In order to obtain the micropellets with the optimum size distribution, it was necessary to syphon out the hot water from the outer jacket of the manufacturing vessel immediately.

Mode of crosslinking :

The mode of crosslinking had very pronounced effect on the physical properties of the micropellets. As three modes

of crosslinking were adopted, the principles and the effects could be discussed as follows :

Vapour crosslinking :

For this method of gelatin crosslinking, some special precautions should be taken during the manufacturing process. The temperature after formation of the embryonic micropellets was maintained below $5^{o}C$. Since the micropellets contained high amount of water, there is every possibility of the micropellets being aggregated on increasing the temperature. The dried micropellets must be maintained exposed to the vapour for uniform crosslinking all over. However, this process could be very useful for drugs which are otherwise soluble in the crosslinking agents.

Crosslinking with aldehyde and isopropanol mixture:

Similar precautions, as described in the case of vapour crosslinking of the gelatin should also be adopted for drugs which would not leach out in the aldehyde or isopropanol crosslinking.

In situ crosslinking

This method offers advantage over the previous two methods in respect of the control of temperature after the formation of the micropellets, since the crosslinking agent was directly added to the system there was no requirement for the water abstracting agent. Therefore, the alcohol soluble drugs could be micropelleted by this method. On the other hand, the aldehyde soluble drugs could not be micropelleted by this process.

Physical Characterization of the Micropellets

Yield :

Table 1 shows that the variable yield was obtained depending on the drug : gelatin ratio and the method of crosslinking of the gelatin matrix of the micropellets, where the later factor appeared to have the most important role.

Increasing the drug : gelatin ratio favoured the degree of yield. In the case of *in situ* crosslinking, the average yield was satisfactory.

Scanning Electron Micrograph :

The scanning electron micrograph in fig. 1 revealed spherical micropelletes with rough surfaces. At higher magnifications, surface shrinkages and pores were observed. After the dissolution study, the micropellets did not change in shape which indicates that the drug was diffused out through the minor pores and channels.

Content uniformity :

As revealed from table 1, the content uniformity of the successive batches was reproducible and within the limit of 5% of the average value. It was observed that significant amount of drug was lost during the process of *in situ* crosslinking which could be explained as the drug leaching as well as diffusion is facilitated when the matrix is soft and contains large amount of water.

TABLE - 1

PHARMACEUTICAL PROPERTIES OF TRIMETHOPRIM MICROPELLETS*

Crosslinking Agent	Parameter	Method of Crosslinking									
		Duration of Crosslinking, hr.							*In situ* Conc. of agent		
		With Isopropanol				Vapour					
		24	48	72	92	48	72	92	3%	5%	10%
Glutaraldehyde	Yield %	85.76	83.21	84.95	84.56	85.24	86.75	84.19	92.72	94.52	93.38
	Content Uniformity %	80.14	82.91	80.75	79.24	84.29	85.58	85.04	76.71	79.24	81.92
Formaldehyde	Yield %	86.98	84.79	85.27	85.99	85.67	85.42	86.97	94.74	95.21	93.81
	Content Uniformity %	80.07	81.14	82.95	83.77	85.97	86.41	85.28	78.55	77.16	76.08

* Total product considered, irrespective of batch size.

Drug : Gelatin ratio 3 : 10

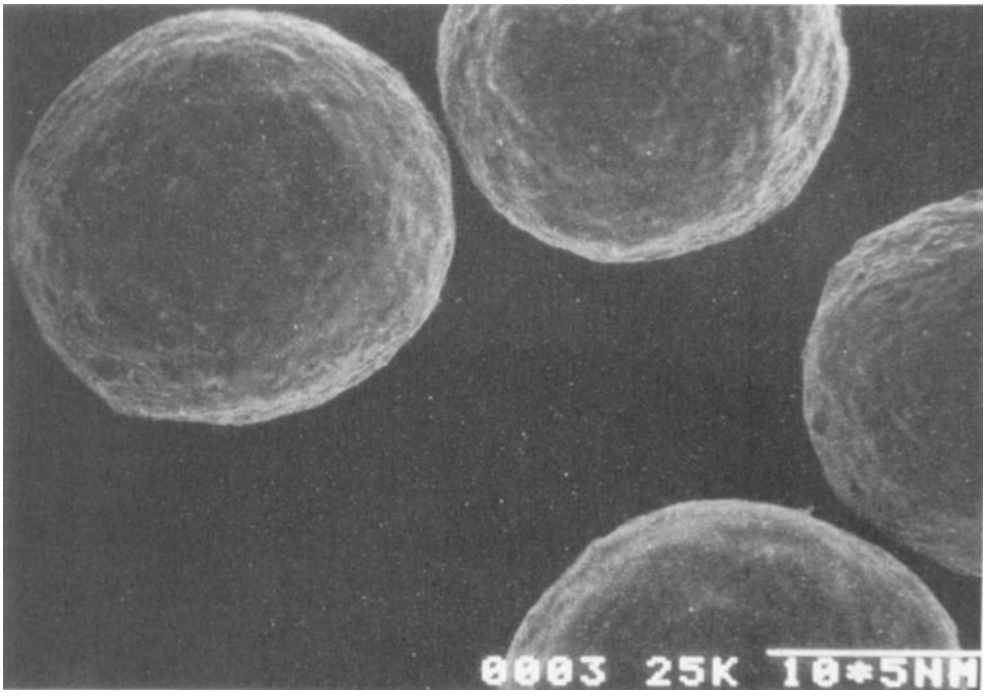

FIGURE 1

Scanning electron micrograph of crosslinked gelatin micropellets showing surface topography

Particle Size Distribution :

As evidenced from table 2, the particle size distribution was influenced by the method of crosslinking of the gelatin matrix. The crosslinking methods other than *in situ* ones are associated with the particle size reduction by the impact with the blade of the stirrer till the completion of the process whereas the *in situ* crosslinking offers the advantages of rigidization of the gelatin matrix while solidification of the micropellets.

TABLE - 2

PARTICLE SIZE DISTRIBUTION OF TRIMETHOPRIM MICROPELLETS CROSSLINKED WITH GLUTARALDEHYDE

Drug:Gelatin Ratio	Method of Crosslinking	Wt. % Retained by Sieves*				
		20	32	44	60	80
3:10	10% Glu-Isoprop	8.57	10.21	47.82	15.69	12.29
	Glu-Vapour	11.25	14.91	51.43	10.87	9.62
	In-situ	9.17	11.28	57.32	12.94	9.47
1:2	10% Glu-Isoprop	13.32	15.87	49.36	11.28	8.49
	Glu-Vapour	14.19	16.52	56.94	7.42	3.91
	In-situ	12.71	14.92	59.19	6.57	5.21
1:1	10% Glu-Isoprop	18.29	20.41	57.14	2.17	1.49
	Glu-Vapour	19.32	21.74	58.47	0.21	0.14
	In-situ	18.43	21.31	58.19	1.14	0.62

* Average of six results.

In Vitro Dissolution Study

As evidenced from figure 2, it is obvious that the drug release rate was dependent on the initial amount of the drug present in the micropellets. Considering the dose size, the optimum drug : gelatin ratio for trimethoprim was found to be 3:10. This optimum proportion resulted in the maximum release of the drug for 5 hours.

It was observed from the fig. 3, that the larger particles of 20/32 mesh size failed to release the total drug within the scheduled period of experimentation. The drug release rate increased with the decreased particle size. In the case of smaller particles, the diffusional path length is less. Therefore, the drug release takes place uniformaly from the surface as well as from the core. Hence the total release at the end of the study and individual release rate both found to be higher in the case of higher drug gelatin ratio. The optimum particle size was chosen to be 32/44 mesh, since this size range appeared to be most suitable for uniform flowability for filling of micropellets in capsules.

The crosslinking of the gelatin micropellets with 10% glutaralhyde/formaldehyde-isopropanol mixture was observed to be most effective in controlling the drug release as shown in fig. 4. Optimum time of exposure to the crosslinking agents was found to be 72 hours. This method offers the unique advantage of uniform crosslinking over all the surface.

The method of crosslinking utilizing glutaraldehyde/formaldehyde vapour has most probably been used widely for crosslinking the gelatin. Since vapour crosslinking is a very slow process as well as all the surfaces are not equivalently

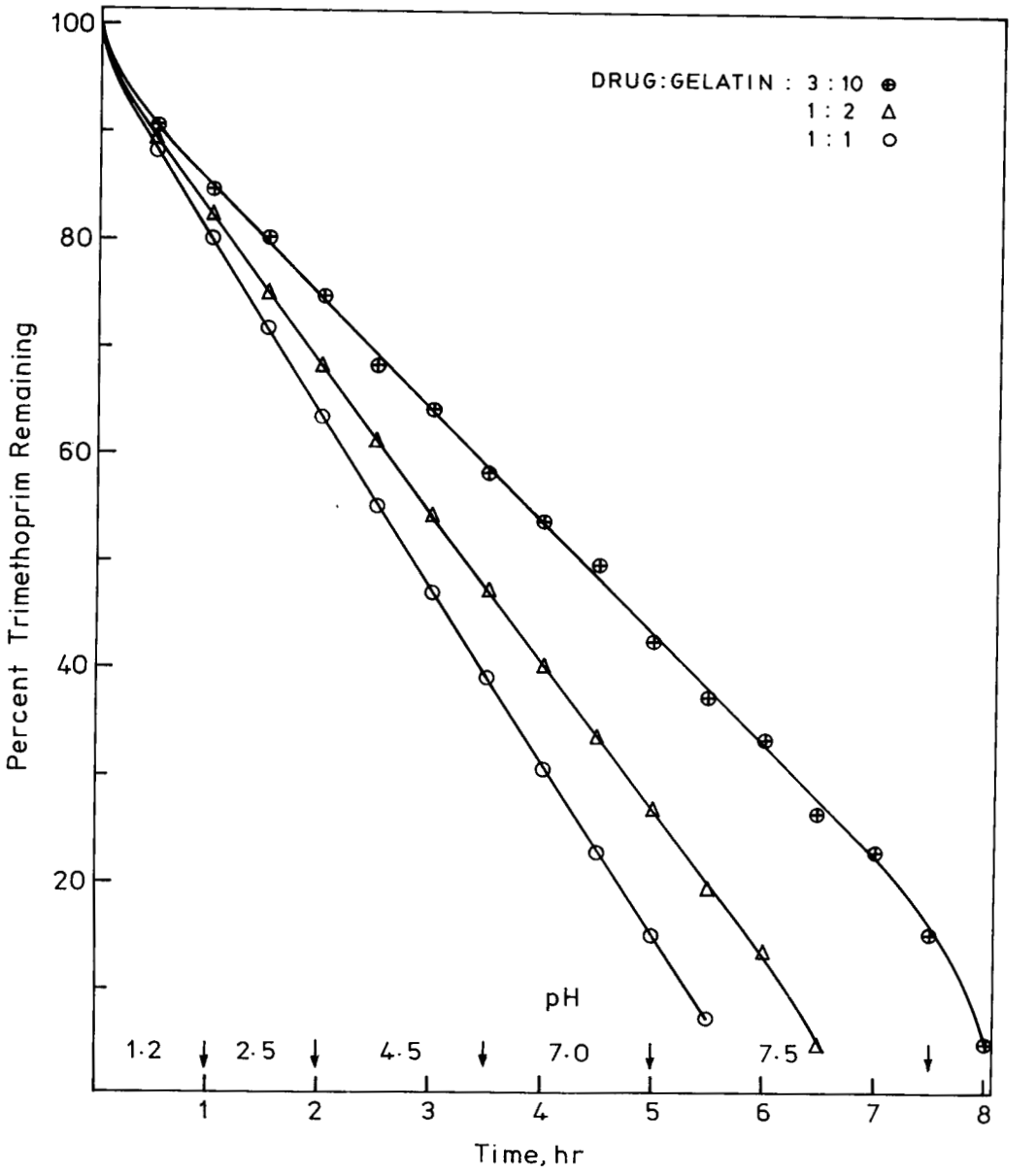

FIGURE 2

Effect of drug : gelatin ratio of micropellets on *in vitro* dissolution profile of trimethoprim ; sieve size 32/44, cross-linking agent 10% glutuaraldehyde - isopropanol mixture for 72 hours

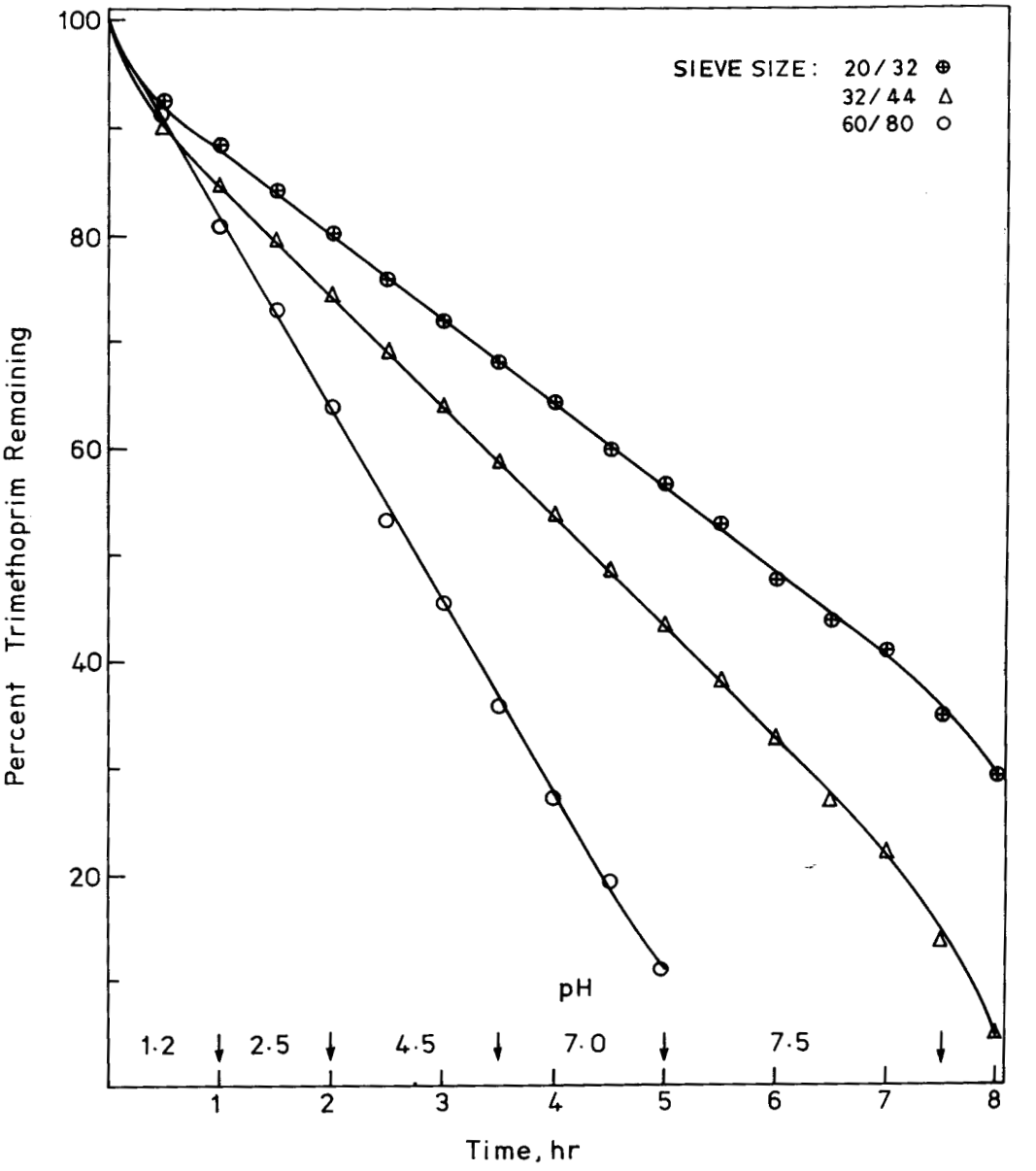

FIGURE 3

Effect of particle size of micropellets on *in vitro* dissolution profile of trimethoprim; sieve size 32/44, crosslinking agent 10% glutaraldehyde - isopropanol mixture for 72 hours

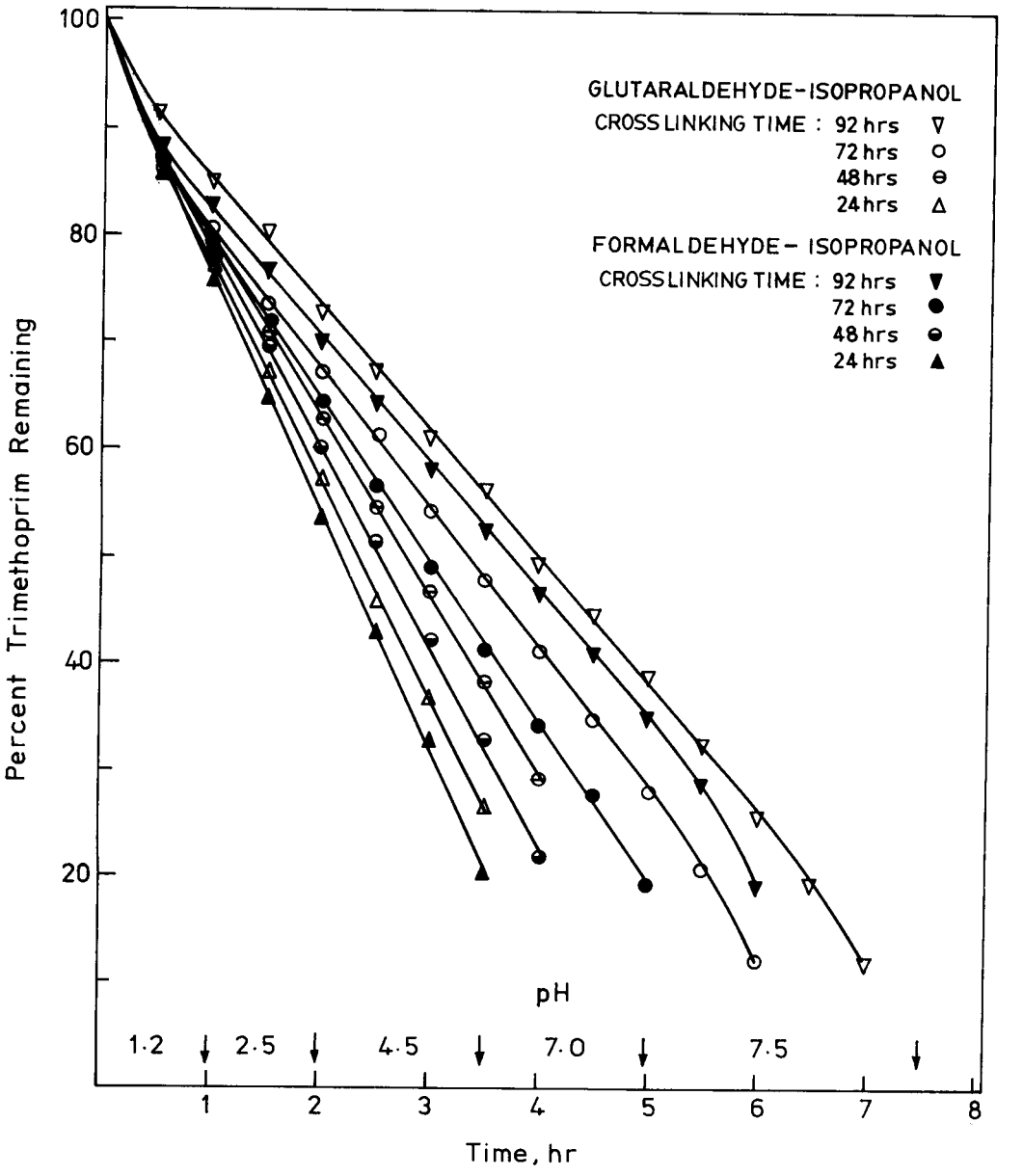

FIGURE 4

Effect of 10% glutaraldehyde/formaldehyde - isopropanol mixture crosslinking of micropellets on *in vitro* dissolution profile of trimethoprim; sieve size 32/44, drug : gelatin ratio 1 : 2

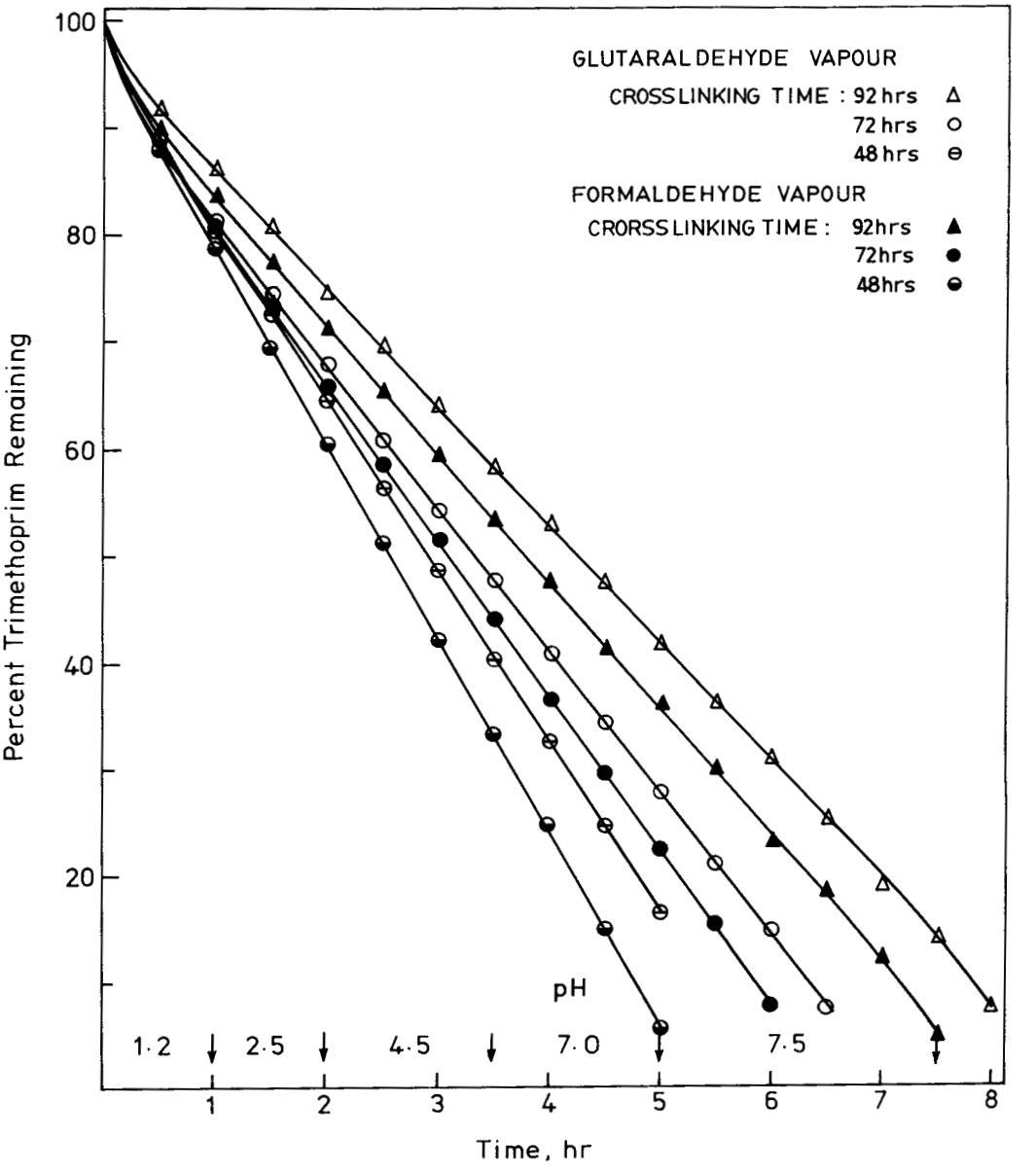

FIGURE 5

Effect of glutaraldehyde/formaldehyde vapour crosslinking of micropellets on *in vitro* dissolution profile of trimethoprim sieve size 32/44, drug : gelatin ratio 1 : 2

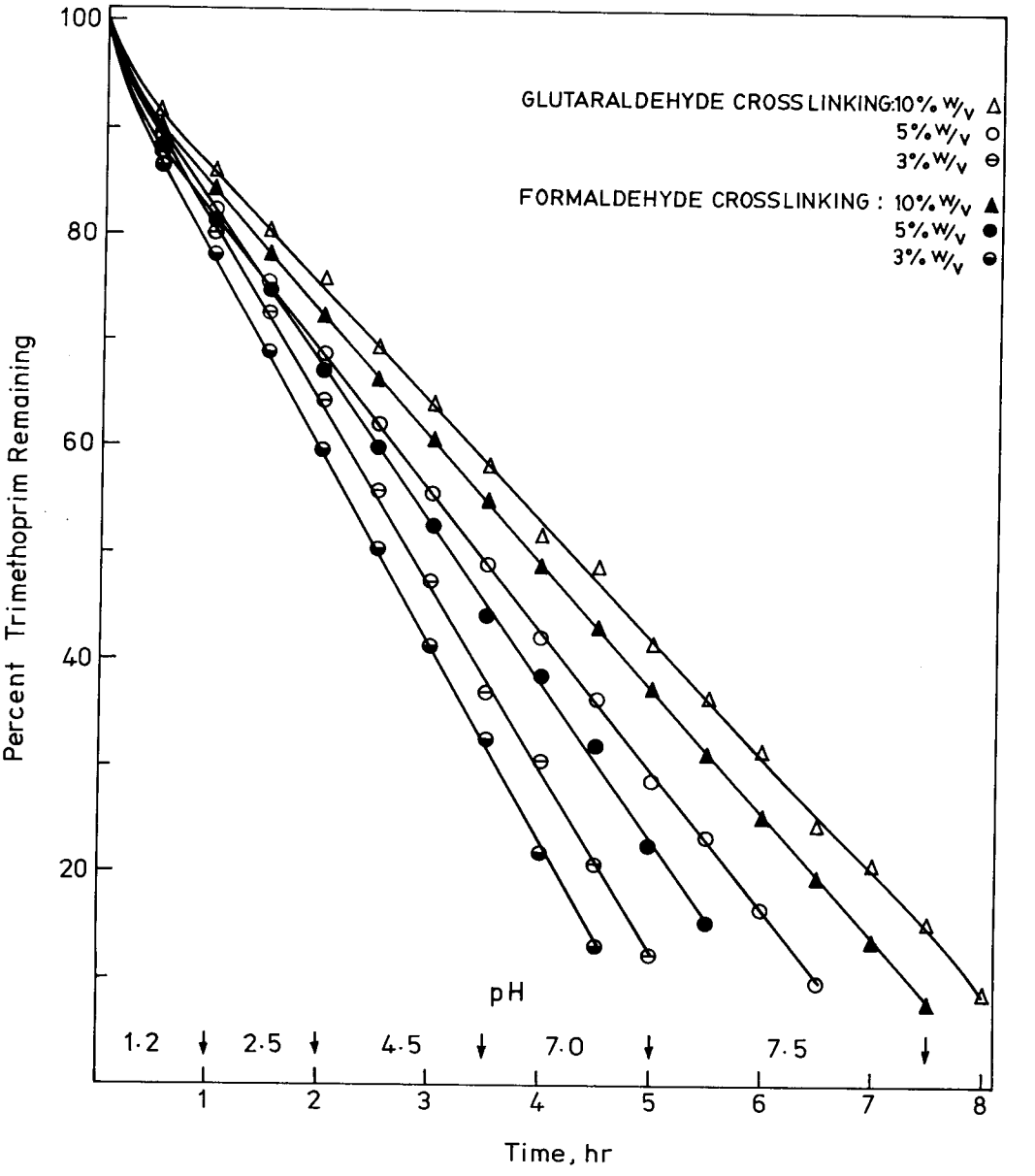

FIGURE 6

Effect of *in situ* rigidization of micropellets with glutaraldehyde/formaldehyde on *in vitro* dissolution profile of trimethoprim ; sieve size 32/44, drug : gelatin ratio 1 : 2

TABLE - 3

KINETIC DATA FOR IN VITRO RELEASE OF TRIMETHOPRIM FROM MICROPELLETS

Cross-linking Agent	Particle Size	Drug-Gelatin ratio	Method of Crosslinking	Duration/ Conc.	t50% (min)	$-Kx10^2$ (min^{-1}) (First Order)
Glutaral	32/44	1:1	A	72 hrs	168	0.412
Glutaral	32/44	1:2	A	72 hrs	198	0.350
Glutaral	32/44	3:10	A	72 hrs	261	0.266
Glutaral	20/32	1:2	A	72 hrs	345	0.201
Glutaral	60/80	1:2	A	72 hrs	165	0.420
Glutaral	32/44	1:2	A	92 hrs	240	0.289
Glutaral	32/44	1:2	A	48 hrs	165	0.420
Glutaral	32/44	1:2	A	24 hrs	141	0.491
Glutaral	32/44	1:2	B	92 hrs	255	0.271
Glutaral	32/44	1:2	B	72 hrs	198	0.350
Glutaral	32/44	1:2	B	48 hrs	168	0.412
Glutaral	32/44	1:2	C	10% w/v	255	0.272

* A - With aldehyde and isopropanol; B - with vapour; C - in situ

Mean of six results.

TABLE - 3 (Contd)

KINETIC DATA FOR IN VITRO RELEASE OF TRIMETHOPRIM FROM MICROPELLETS

Cross-linking Agent	Particle size	Drug:Gelatin ratio	Method of Crosslinking	Duration/ Conc.	t50% (min)	$-Kx10^2$ (min^{-1}) (First order)
Glutaral	32/44	1:2	C	5% w/v	204	0.339
Glutaral	32/44	1:2	C	3% w/v	165	0.420
Formal	32/44	1:2	A	92 hrs	225	0.308
Formal	32/44	1:2	A	72 hrs	180	0.385
Formal	32/44	1:2	A	48 hrs	150	0.462
Formal	32/44	1:2	A	24 hrs	132	0.525
Formal	32/44	1:2	B	92 hrs	225	0.308
Formal	32/44	1:2	B	72 hrs	183	0.379
Formal	32/44	1:2	B	48 hrs	153	0.453
Formal	32/44	1:2	C	10% w/v	228	0.304
Formal	32/44	1:2	C	5% w/v	192	0.361
Formal	32/44	1:2	C	3% w/v	150	0.462

* A - With aldehyde and isopropanol; B - with vapour; C - in situ

Mean of six results.

exposed to vapour, this method produced the micropellets with surfaces of frequent non-crosslinking areas which are more prone to form channels to leach out the drug causing much less prolongation of the drug release profile as shown in fig. 5. The effect of rigidization was much less than the other two methods discussed, because the tighter structure produced in the matrix makes it difficult for crosslinking agent to penetrate effectively.

As evidenced from the fig. 6, the *in situ* crosslinking of the gelatin matrix renders higher drug release rates because the crosslinking of gelatin occurs when the matrix is a soft gel and imbibed much water which is evaporated during the process of drying forming channels in the matrix, rendering the matrix easily swellable in contact with water. Therefore, the drug is much easily diffused from the spongy matrix than in the process with crosslinking agent - isopropanol. During the drying process, the surface shrinkage is also expected to occur.

Kinetics of drug release :

The drug release kinetics was evaluated using the RELAN[12] computer package. The drug release profile depicts the first order kinetics upto 80% of the total drug content. The drug release data was interpreted using the linear regression and subsequent statistical analysis. The t 50% values are depicted in table 3. The drug release is explained to occur by diffusion controlled first order dissolution, where the following events are expected to occur in seriatim;

- dissolution of the drug adsorbed on the surface of the micropellets and thereby giving rise to

the phenomenon of bursting effect and initial depression of the plots,

- permeation of the solvent molecules through the pores and channels of the matrices,
- solvation of the drug molecules,
- diffusion of the solvated drug molecules to the surface,
- diffusion of the solvated drug to the bulk of the dissolution medium.

The residual formaldehyde and glutaraldehyde in the micropellets were determined by the method of Macfadyen[13] and was observed to be within 5 p.p.m., described as non-toxic[14].

In conclusion, crosslinked gelatin micropelletization would be very effectively applicable as attempts are being made to prolong the release of drugs. Crosslinking of the gelatin matrices with glutaraldehyde-isopropanol mixture offers most acceptable optimum drug release profile.

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