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International Journal of Pharmaceutics 144 (1996) 209–216

international journal of pharmaceutics

In vitro release of theophylline from cross-linked gelatin capsules

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Received 23 July 1996; accepted 13 September 1996

Abstract

Hard gelatin capsules were manufactured in our laboratory and cross-linked with terephtaloyle chloride before being filled with pure theophylline powder. Different reaction times were used to carry out the cross-linking process, either before or after drying of capsules. The in vitro drug release was carried out on comparison with commercial and laboratory-made conventional hard gelatin capsules and with Dilatrane®. Release profiles showed that cross-linking before or after drying of capsules allowed sustained release of drug. However, a slower rate of release was shown from capsules cross-linked before drying. The rate of release decreased when the cross-linking reaction time was increased. When the reaction time was of 30 min, capsules exhibited a nearly zero-order release ($r = 0.999$) with a cumulative drug release of $63.24 \pm 10.48\%$ only. Cumulative drug release, $DT_{50\%}$ and DE from capsules cross-linked before drying had nearly the same release characteristics as Dilatrane®. Cross-linking before and after drying of capsules permitted showing up the influence of moisture on the cross-linking density and, therefore, on the rate of drug release. Copyright © 1996 Elsevier Science B.V.

Keywords: Gelatin cross-linking; Hard gelatin capsule; Sustained release; Theophylline

1. Introduction

Theophylline is a xanthine bronchodilator. It relaxes directly the smooth muscle of the bronchial airways and pulmonary blood vessels. So, theophylline is used to relieve and/or prevent symptoms of asthma. It has a narrow therapeutic range and its short half-life is influenced by a number of known variables such as chronic alcoholism, impaired hepatic and renal failure, age, etc. Thus, large fluctuations in theophylline blood concentrations following oral administration of the drug in conventional dosage forms are frequently reported. Therefore, theophylline has re-

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PII S0378-5173(96)04757-6

ceived a considerable amount of attention in sustained release formulations. Sustained release dosage forms provide more uniform serum concentrations with less fluctuations in peak-valley levels and reduce dosing frequency, thereby leading to a better patient compliance (Latha et al., 1995). So, many theophylline sustained release dosage forms have been developed like ethylcellulose microcapsules (Amperiadou and Georgarakis, 1995; Chen et al., 1995), microencapsulated ion-exchange resins (Motycka et al., 1985; Moldenhauer and Nairn, 1990), Gelucire® matrix granules in hard gelatin capsules (Brossard et al., 1991), poly(lactide) pellets (Bodmeir and Chen, 1989), insoluble matrix of microcristalline cellulose (Peh and Yuen, 1995) and hydroxypropylmethylcellulose hydrocolloïd matrice (Möckel and Lippold, 1993). Cross-linked materials as casein microspheres (Latha et al., 1995), chitosan microspheres (Thanoo et al., 1992) and polyvinyl alcohol microspheres (Thanoo et al., 1993) have also been used. All reported results showed that in vitro release of theophylline from these dosage forms varied with many formulations and parameters. However, in spite of the wide variations in their release properties, they appeared to be more suitable in the controlled release of drug than enteric coating dosage forms. Futhermore, the enteric coating is not the best choice for patients whose fasted gastric pH is superior to 5 (Russell et al., 1993). This is especially true when a high level of enteric coating of hard gelatin capsule is required to maintain enteric protection during a prolongated gastric residence time (Fremstad et al., 1979; Burns et al., 1994). On the other hand, the cross-linking process causes formation of a rubbery, water-insoluble but swollen membrane during dissolution testing (Digenis et al., 1994). This membrane acts as a controlled release barrier. So, the cross-linking process was used by many authors in the development of controlled release dosage forms (Benita et al., 1984; Lévy and Andry, 1987; Digenis et al., 1994; Latha and Jayakrishnan, 1994; Lou and Groves, 1994; Narayani and Panduranga Rao, 1994; Latha et al., 1995) to be administered either by oral or by parenteral route.

The purpose of this work, therefore, was to prepare theophylline hard gelatin capsules cross-linked by terephtaloyle chloride, and to study the influence of the gelatin cross-linking reaction time on the rate of theophylline release. This 'sustained' release dosage form is easy to produce simply by filling active substances in the cross-linked wall capsules.

Theophylline was chosen for its pharmacokinetics and as a model of drug poorly soluble in water.

2. Materials and methods

2.1. Materials

Gelatin of type B from animal bones (pH 5.69, bloom strength 251 g and viscosity 4.75 mPa for moisture 11.05% and concentration 6.67%) was gift from SBI (Systems Bio-Industries, Boulogne Billancourt, France), terephtaloyle chloride was purchased from Sigma Chemical (St. Louis, USA), theophylline and transparent empty hard gelatin capsules were obtained from Cooper (Melun, France). All other chemicals and solvents employed were of analytical grade and used as received without futher purification.

Stainless steel pins were manufactured in our engineering works.

2.2. Methods

2.2.1. Preparation of hard gelatin capsules

Hard gelatin shells were prepared by a laboratory process using ten pairs of pins (No. 0) mounted over a holding plate. Pins were dipped into a warm gelatin sol to form caps and bodies simultaneously. Gelatin sol contained 33.30% of gelatin, 0.15% of methylparaben as preservative and 66.55% of deionized water. This solution was maintained at 57 ± 1°C using a water bath thermostatically controlled.

2.2.1.1. Conventional gelatin capsules. The pins, previously lubricated with mineral oil and stored at ambient temperature (22 ± 1°C), were dipped to the desired height level into warm gelatin solu-

tion. After that, they were pulled out and rotated to avoid excessive local concentrations of gelatin. All capsule parts placed on the pin were gently dried in a temperature and humidity controlled oven. Finaly, hard gelatin shells were manually stripped from the pins.

2.2.1.2. Cross-linked gelatin capsules (CGC). Cross-linked gelatin capsules were prepared according to the process described above for conventional capsules, but capsules were dipped into a 3% terephtaloyle chloride solution in a chloroform-cyclohexan (1:4) mixture at laboratory temperature, during various given times (0.25, 1, 7.5 and 30 min), either before or after drying of capsules. Cross-linked capsules were then washed several times with cyclohexan and dried as the conventional capsules in the same drying oven. After drying, shells were manually stripped from the pins after cross-linking.

All capsules, conventional or cross-linked, were finally filled with 20 mg of theophylline (particle size ≤ 250 μm).

2.2.1.3. Reference gelatin capsules. Two types of commercial reference capsules were used in this work: empty gelatin capsules and capsules of Dilatrane®. The empty transparent hard gelatin capsules (No. 0) were filled with 20 mg of theophylline, in order to compare release characteristics of the commercial shells with our laboratory-made non cross-linked shells. The theophylline sustained release dosage form 'Dilatrane®' (Laboratoires Pharbiol, Tassin la demi-lune, France) contained microspheres in hard transparent capsules and was used in order to compare the sustained release characteristics of our cross-linked capsules with the commercial product. The content of hard gelatin capsule (No. 0) of Dilatrane® was reduced to an equivalent of 20 mg of theophylline.

2.2.2. Dissolution studies

The in vitro release from capsules was determined by dissolution test using the USP XXIII basket apparatus at 37°C (Sotax AT7, Basel, Switzerland). The basket containing one capsule was rotated at 100 r.p.m. in 1000 ml of HCl 0.1 N (pH 1.2) for 1 h. Afterwards, in the case of cross-linked capsules and Dilatrane®, HCl medium was quickly withdrawn and replaced with an equal volume of phosphate buffer solution (pH 6.8) and rotation was continued for another 7 h.

The dissolution medium was continuously pumped, filtered through 10 μm pore size polypropylene filter and then passed through the continuous flow cell of a spectrophotometer (Beckman 34, USA). The cumulative absorption/time curves were measured at 271 nm. Results were computed with a standard calibration curve of the drug ($r = 0.999$) and dissolution kinetics were measured from the mean of six determinations.

2.2.3. Statistical analysis

Statistical analysis of drug release was performed using one-way analysis of variance (ANOVA). A Scheffé test was applied to the experimental values. A significance level of $P = 0.05$ was chosen. The Scheffé method was interesting to carry out after ANOVA, since it permitted a posteriori comparison of multiple results and isolation of the sources of significant differences (Scheffé, 1953). Statistical analysis of dissolution efficiency and dissolution halftime was carried out with Student's t-test.

3. Results and discussion

3.1. Manufacturing of capsules

The main characteristics of laboratory-made capsules and those of commercial capsules are shown in Table 1.

Laboratory-made hard gelatin capsules were of 20 ± 1 mm and 12 ± 1 mm in length for the body and cap respectively and of 0.35 ± 0.05 mm thick. In the other hand, their final moisture content was of $11 \pm 1\%$. Our conventional hard gelatin capsules were desintegrated after about 13 min.

Non cross-linked gelatin capsules were more stiff and lightly less transparent than commercial capsules. That is likely due to the gelatin used in the formulation of laboratory-made capsules and also to the manufacturing conditions. Commercial

hard gelatin capsules are usually a mixture of pork skin gelatin (bringing plasticity and clarity) and bone gelatin (bringing firmness) whereas only the last one was used in the manufacturing of our laboratory-made capsules.

CGC had the same characteristics as conventional capsules but they were slightly transparent. Cross-linking reaction was carried out before stripping capsules from pins and therefore only the external face of capsules was concerned. On the other hand, no appreciable technical difficulties were encountered, whereas considerable technical problems were reported when aqueous dispersions were used in the hard gelatin capsules coating (Plaizier-Vercammen et al., 1992). During coating with aqueous formulations, water can induce gelatin softening and shells become sticky, or gelatin shell can become brittle due to water evaporation and drying (Plaizier-Vercammen et al., 1992). In addition, when gelatin cross-linking is conducted in organic medium (chloroform/cyclohexan mixture in our case), the reaction is mainly limited to the exposed material surface (Latha et al., 1995).

3.2. Release kinetics

The mean drug release profiles from conventional laboratory-made and commercial capsules are shown in Fig. 1. The drug release from both types of capsules was rapid and complete within 42 min.

Table 1
Main physical characteristics (mean ± S.D.) of conventional laboratory-made and commercial hard capsules

Parameters	Laboratory-made capsules	Commercial capsules
Mean length body (mm)	20.0 ± 0.4	18.8 ± 0.2
Mean length cap (mm)	12.0 ± 0.3	11.1 ± 0.1
Thickness (mm)	0.35 ± 0.05	0.2 ± 0.04
Moisture (%)	11 ± 1	14 ± 1
Desintegration time (min)	13 ± 1	2.5 ± 0.5

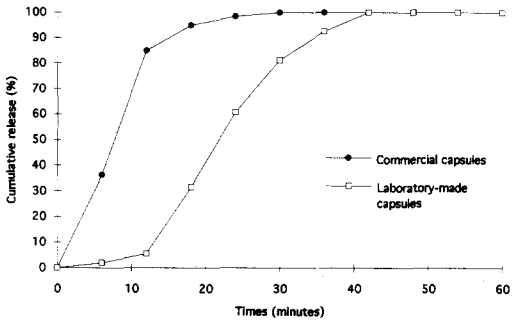

Fig. 1. Mean release profile of theophylline from conventional commercial and laboratory-made hard gelatin capsules.

However, drug release from laboratory-made capsules which was very slow at the beginning, increased quickly after 12 min, whereas drug release from commercial gelatin capsules was very rapid from the beginning and became complete within 30 min. The time course of 100% drug dissolution was significantly different ($P < 0.01$) between the two types of capsules and could be explained by the 2-fold shell thickness for the laboratory-made capsule as compared to the commercial one. When cumulative drug release attained 100% from commercial capsules, only 81.25 ± 16.14% of the initial drug amount was released from laboratory-made capsules. Mean dissolution efficiency (DE) from commercial and laboratory-made capsules was 72.87 ± 5.75% and 28.09 ± 13.76%, respectively, showing a better extent of dissolution in vitro.

The CGC released theophylline, as gelatin cross-linked microspheres (Forni et al., 1989), by diffusion on through the cross-linked gelatin membrane without any apparent desintegration (Digenis et al., 1994). Body and cap of the capsule were never separated during the 8 h of the time course test but turned into on elastic mass of gelatin in which body and cap were not differentiated. Forni et al. (1989) had already reported a similar swelling process for gelatin microspheres glutaraldehyde cross-linked.

Fig. 2 shows the mean drug release profiles from Dilatrane® and various cross-linked gelatin capsules before drying. The main characteristics of in vitro release of theophylline from CGC and Dilatrane® are given in Table 2 and statistical comparison (P values) are represented in Table 3.

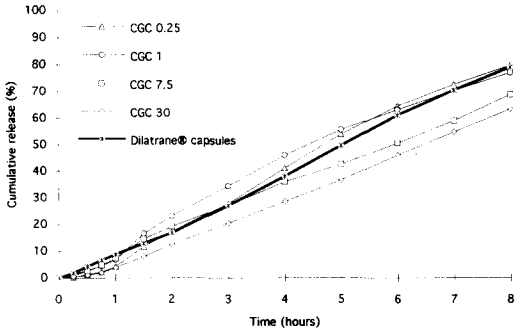

Fig. 2. Mean release profiles of theophylline from cross-linked gelatin capsules (CGC) and Dilatrane® capsules.

Whatever the examined CGC, theophylline was released very slowly in acidic medium. The mean cumulative drug release at pH 1.2 was significantly different between 30 min CGC and Dilatrane®, but not between the CGC. Consequently, cross-linking of gelatin led to a gastroresistance of capsules but it was not always an adequate enteric coating since 3.84–7.40% of the initial drug amount was released in 1 h in acidic medium.

After complete change of acidic dissolution medium with the pH 6.8 phosphate buffer, the dissolution rate of Dilatrane® remained unchanged whereas the dissolution rate increased suddenly from all CGC. Moreover, this burst effect release was more remarkable from capsules with a short time of cross-linking reaction (0.25 and 1 min) and was likely a function of the cross-linking extent which depends among other parameters on the reaction time.

In the cross-linked gelatin, the reversibility of aminal reactions is pH dependent and aminal decomposition is reversible under basic conditions (Digenis et al., 1994). So, the increased drug release when acidic medium was replaced with a neutral medium could be explained by a partial decomposition of the cross-linked gelatin at pH 6.8 owing to the inevitable increase in the rate of release which became more uniform after 1.5 or 2 h. A similar burst effect release of theophylline from poly(lactide) pellets, after that 0.1 M HCl medium was replaced with pH 7.4 buffer, has been reported by Bodmeir and Chen (1989). It was attributed to the character of low molecular weight poly(lactide) which may act as an enteric material. However, from poly(lactide) pellets, the remaining drug was released rapidly at pH 7.4. This gastroresistance was also found with cross-linked gelatin microcapsules (Lévy and Andry, 1987). In addition, these authors have found that extent of cross-linking of gelatin was lesser with type B than type A and therefore the gastroresistance of that cross-linked gelatin was lower.

At pH 6.8, whatever the cross-linking reaction time, the release of theophylline, as shown in Fig. 2, was similar from the four CGC. The dissolution halftime (DT50%) increased with the cross-linking reaction time from 4.92 ± 1.22 to 6.61 ± 0.95 h when the cross-linking reaction time was 0.25 and 30 min, respectively (Table 2). In the other hand, dissolution efficiency (DE%) decreased as the cross-linking reaction time increased except for 0.25 min. However, largest amount of theophylline released after an 8 h

Table 2
Characteristics (mean ± S.D.) of in vitro release of theophylline from cross-linked gelatin capsules and Dilatrane®

Cross-linking time reaction (min) or reference	Cumulative drug release (%)	$DT_{50\%}$ (h)	DE%
0.25	79.58 ± 15.49	4.92 ± 1.22	40.12 ± 11.31
1	76.85 ± 8.98	4.55 ± 0.77	42.24 ± 5.43
7.5 (bd)[a]	68.75 ± 15.90	5.88 ± 1.68	34.47 ± 12.10
7.5 (ad)[b]	89.71 ± 7.85	3.18 ± 1.21	51.10 ± 8.14
30	63.24 ± 10.48	6.61 ± 0.95	29.20 ± 5.07
Dilatrane®	78.81 ± 10.84	5.16 ± 1.01	38.84 ± 8.76

$DT_{50\%}$, Dissolution halftime.
DE, Dissolution efficiency.
[a]Before drying.
[b]After drying.

Table 3
Statistical comparison of characteristics (P values) of in vitro release of theophylline from cross-linked gelatin capsules and Dilatrane® containing 20 mg of drug

Preparations		Cumulative theophylline release (%)[a]	$DT^{b}_{50\%}$	$DE\%^{b}$
CGC30	vs Dilatrane®	$P<0.05$	$P<0.05$	$P<0.05$
	vs CGC 7.5bd	N.S.	N.S.	N.S.
	vs CGC 1	$P<0.05$	$P<0.05$	$P<0.05$
	vs CGC 0.25	$P<0.05$	$P<0.05$	N.S.
CGC 7.5bd	vs Dilatrane®	N.S.	N.S.	N.S.
	vs CGC 1	$P<0.05$	N.S.	N.S.
	vs CGC 0.25	N.S.	N.S.	N.S.
	vs CGC 7.5ad	$P<0.01$	$P<0.005$	$P<0.01$
CGC 1	vs Dilatrane®	N.S.	N.S.	N.S.
	vs CGC 0.25	N.S.	N.S.	N.S.
CGC 0.25	vs Dilatrane®	N.S.	N.S.	N.S.
CGC 7.5ad	vs Dilatrane®	$P<0.05$	$P<0.01$	$P<0.05$

$DT_{50\%}$, dissolution halftime.
DE%, dissolution efficiency.
CGC 30, cross-linked gelatin capsules 30 min.
CGC 7.5bd, cross-linked gelatin capsules 7.5 min before drying.
CGC 7.5ad, cross-linked gelatin capsules 7.5 min after drying.
CGC 1, cross-linked gelatin capsules 1 min.
CGC 0.25, cross-linked gelatin capsules 0.25 min.
[a]ANOVA with Scheffé test.
[b]Student's t-test.

course time was found from 0.25 min CGC. We have not the plausible explanation of this observation.

Cumulative theophylline release, $DT_{50\%}$ and DE% values were significantly different between 30 min CGC and Dilatrane® and between 1 and 30 min CGC.

At 8 h after the beginning, the release of drug was not yet complete. The DE% and $DT_{50\%}$ of Dilatrane® were nearly the same as those of 0.25 and 1 min CGC. This result suggests that a short cross-linking time may be enough to control the in vitro drug release from gelatin capsules.

It is of interest to note that, in the two dissolution media, 7.5 and 30 min CGC exhibited almost zero-order drug release up to a cumulative theophylline release percentage of 68.75 ± 15.90 and 63.24 ± 10.48, respectively (Table 2). The coefficients of correlation calculated between zero and 8 h had the same value (0.9983) for 7.5 and 30 min CGC and was of 0.9991 for Dilatrane®. When these coefficients were determined between 1 and 8 h their values were, respectively, of 0.9984, 0.9998 and 0.9991. It is then clear that, as in coating process, release rate can be easily controlled by a cross-linking process. So, it is likely that drug release resulted from two simultaneous mechanisms: the diffusion through the cross-linked membrane as decribed above and the reversibility of the cross-linking reaction. On the other hand, the diffusion mechanism was probably more important when the cross-linking reaction time was larger than 1 min resulting in higher cross-linking density. A sustained drug release has been already reported (Lévy and Andry, 1987) from sodium salicylate loaded cross-linked gelatin microcapsules but the rate of the in vitro release was low enough to be used in the design of sustained release dosage forms. The nearly zero-order release seen from 30 min CGC was similar to that reported by Narayani and Panduranga Rao (1994) about methotrexate release from glutaraldehyde cross-linked gelatin microspheres.

As shown in Fig. 2, the rate of release varied with the cross-linking reaction time, which influences the cross-linking density. Consequently, the

small difference between the rate of release from 7.5 and 30 min CGC was probably due to a little difference between the cross-linking densities in both types of capsules. These findings are in agreement with results reported by Latha et al. (1995) about the release of theophylline from glutaraldehyde cross-linked casein microspheres.

Release profiles of theophylline from hard gelatin capsules which were cross-linked, during 7.5 min, before or after drying are shown in Fig. 3. The rate of drug release in acidic medium was nearly the same for both types of capsules. Just after the complete change of the acidic medium with the pH 6.8 medium, the burst effect release appeared. This effect was more remarkable and drug release became more rapid when capsules were cross-linked after have been dried. However, in both cases, 8 h after the begining of the test, drug release remained incomplete. The cumulative drug release from capsules cross-linked before ($68.75 \pm 15.90\%$) or after ($89.71 \pm 7.85\%$) drying were significantly different ($P < 0.01$). The differences between $DT_{50\%}$ values and between the DE% values from the two types of capsules were significant ($P < 0.01$ and $P < 0.005$, respectively). These results are in agreement with those already reported by Digenis et al. (1994), proving that humidity strongly influences the rate of the cross-linking reaction. So, cross-linking density was much lower after than before drying of capsules and, therefore it was normal that drug release rate is lower.

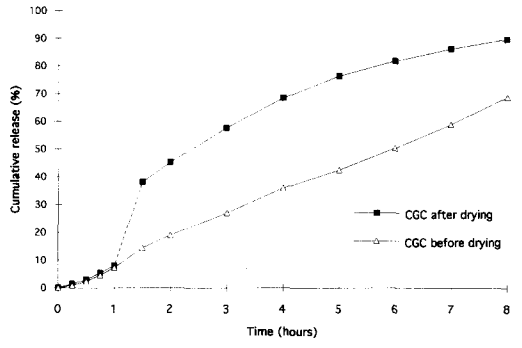

Fig. 3. Mean release profiles of theophylline from gelatin capsules (CGC) cross-linked before or after drying.

4. Conclusion

The results reported in this work proved that cross-linking of gelatin hard capsules with terephtaloyle chloride could be of interest to control drug release by direct filling of cross-linked capsules with drug. Cross-linking reaction time before drying of capsules appeared to be suitable to obtain a nearly in vitro zero-order release. However, more in vitro and in vivo investigations must be undertaken to confirm the potential of the cross-linked gelatin capsules in the design of sustained release oral dosage forms. These studies are in progress.

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