# A STUDY OF THE INFLUENCE OF CROSSLINKING DEGREE ON THE PHYSICOCHEMICAL PROPERTIES OF GELATIN MICROPARTICLES

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The application of the microencapsulation technique in medicine and pharmacy has been extensively studied in recent years. The microencapsulation of small particles in polymeric, waxy or other kind of protective shells has become a well-established technology for coating and isolating various substances.

The present paper presents the results obtained in a study concerning the influence of crosslinking degree on the physicochemical properties of gelatin microparticles containing xantinol nicotinate. The microparticles have been prepared by emulsifying a gelatin solution in an oily phase (sun flower oil). So far, gelatin microparticles have been widely evaluated as an excellent drug carrier. Nevertheless, gelatin dissolves rather rapidly in aqueous environments, making the usage of the polymer quite difficult for producing extended release delivery systems. Therefore, in obtaining extended release gelatin microparticle formulations, the usage of a crosslinking agent generating non-soluble networks is necessary. Glutaraldehyde was selected as crosslinking agent. The crosslinking process was completed either by maintaining the test microparticles in glutaraldehyde vapors for various periods of time, or by using a 35% glutaraldehyde solution in acetone.

The test gelatin microparticles were characterized *in vitro* in terms of both physical and chemical properties (such as particle size, density, drug content, swelling dynamics and drug release).

The experimental data confirmed that the crosslinking degree significantly influenced drug loading, swelling kinetics, as well as the mechanism of drug release kinetics.

Keywords: microparticles, gelatin, crosslinking, glutaraldehyde, xantinol nicotinate

# INTRODUCTION

The microencapsulation technique has been extensively used to modify the rate and extent of drug release. The main limitation of classical extended release formulations consists in the local accumulation of active substances leading to irritant effects on the gastrointestinal mucosa.

Using microencapsulated drugs, the active substance is widely distributed throughout the gastrointestinal tract, providing increased drug absorption and substantially decreasing the irritant effect.<sup>1</sup>

Various materials, as well as many techniques for the preparation of microcapsules have been developed so far. One of the methods described in literature for obtaining gelatin microparticles is the technique of emulsifying the drug solution with mineral oils (such as sun flower, paraffin or olive oil).<sup>1-5</sup>

Gelatin microparticles have been widely evaluated as drug carriers.<sup>5-8</sup> Gelatin, a natural polymer, is biodegradable, biocompatible, nontoxic, and noncarcinogenic.<sup>6</sup> As gelatin rapidly dissolves in aqueous environments, it can be used as such to obtain systems for long-term delivery. The studies reported in literature have shown that the cross-linked aldehyde gelatin microparticles (by the formation of nonsoluble networks inside the microparticles) have a longacting, biodegradable ability, most suitable for the controlled delivery of numerous drugs.<sup>9,10</sup>

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The test microparticles were produced following and further elaborating the method reported by Tanaka *et al.*<sup>1</sup> The gelatin microparticles were manufactured by emulsifying a solution of gelatin-xantinol nicotinate (XN) in sun flower oil. Further, a fraction of the microparticles was subjected to crosslinking in a saturated atmosphere of a 35% glutaraldehyde solution, previously maintained for 24 hours in a 0.5 L desiccator – before placing the microparticles in it and keeping them for about 24, 48 or 72 h. Subsequently, the microparticles were placed into a solution of glutaraldehyde in acetone (35%) for approximately 2 hours at 10 °C. In order to remove the residual glutaraldehyde, the crosslinked gelatin microparticles were rinsed three times using an aqueous acetone solution (99.5% acetone by volume). Finally, the rinsed microparticles were vacuum-dried for 24 hours in order to evaporate any acetone residue.

The study aimed to investigate the influence of crosslinking degree on the physicochemical properties of xantinol nicotinate gelatin microparticles. Throughout the *in vitro* study, the following properties were evaluated: size, density, drug content, swelling dynamic and drug release.

#### EXPERIMENTAL

#### Materials

• Noncrosslinked gelatin microparticles (microspheres) with XN (NXN);

• XN gelatin microparticles crosslinked for 24 (CXN24), 48 (CXN48) and 72 h (CXN72) in a saturated atmosphere of glutaraldehyde vapors;

• XN gelatin microparticles crosslinked in glutaraldehyde solution (35%) in acetone for 2 h (CXN2SOL).

#### Methods

#### **Density evaluation**

The density of the tested microparticles was analyzed using the pycnometric method.<sup>1,11</sup> The effective determination is based on the following equation:

$$\rho = \frac{(b-a)\rho_1}{(d-a) - (c-b)}$$
(1)

where:  $\rho$  = density of the microparticles

a = weight of the empty pycnometer

b = weight of the microparticle filled pycnometer c = weight of the microparticle and solvent filled

pycnometer

d = weight of the pycnometer when using acetone as solvent ( $\rho_{acetone}^{20^0} = 0.79 \text{ g/cm}^3$ )

#### Particle size

Particle size range and distribution of microparticles were determined using standard sieves, a specially calibrated system, model VEB Metallwebereii Neustadt Orla.

#### X-ray diffractometry

The X-ray diffraction patterns have been recorded using an X-ray diffractometer, model TUR M-61. For this procedure, the parameters were set as follows: Ni filter, K $\alpha_1$  radiation, tube settings 30 kV, 20 mA, and angular speed 1° (20) per minute.

#### Drug content

The drug content of the tested microparticles was determined by spectroscopy at a wavelength  $\lambda = 270.4$  nm, after previously extracting the drug in distilled water at 37 °C and stirring this blend at 200 rpm (2 h).

#### Swelling kinetics

The swelling dynamics of the tested gelatin microparticles was determined using the method previously reported by Robert et al.<sup>12</sup> The dried test microparticles were placed on observation plates equipped with liquid containers and studied with an optical microscope (Zeiss optic microscope). Their initial diameter,  $D_0$ , was recorded and the containers were filled with deionized water. One drop of methylene blue was used in order to observe how the water front penetrates the microparticles. The increase of the swelling diameter,  $D_t$ , due to water transportation through the test microparticles, was observed at the room temperature as a function of time; the process was allowed to take place until the considered microparticles achieved full equilibrium, with  $D_{\infty}$  diameter. Six microparticles with diameters up to 360 µm were tested for each of the studied groups. The mechanism of the water transport in the tested microparticles and the values of the "n" parameter were calculated using Peppas equation, adapted for the swelling process:13

$$\frac{D_t - D_0}{D_0} = kt^n \tag{2}$$

where:

 $D_0$  = size of the microparticles at time 0

 $D_t = size of the microparticles at time t$ 

k = the swelling rate

n = parameter of the transport type of water

The swelling degree (Q) was calculated according to the relation:<sup>7</sup>

$$\frac{D_t}{D_0} = \frac{D_{10}}{D_0} = \frac{D_{\infty}}{D_0} = Q$$
(3)

where:  $D_{\infty}$  = size of the microparticles at equilibrium

#### In vitro dissolution

In order to determine how XN is released from the tested microparticles, an *in vitro* column type device was selected and used.

The medium used for dissolution was distilled water at  $37\pm0.1$  °C temperature, at a flow rate of 2 mL/min. Samples were taken at appropriate intervals, up to 3 hours. The samples were further analyzed by measuring their UV absorbance at a wavelength of 270.4 nm. The drug concentration for each of the sample solution was calculated using a standard curve. The *in vitro* dissolution of XN from the tested microparticles was reported as being the average of six determinations.

In order to understand the kinetics and mechanism of drug release, the results of the *in vitro* study were primarily tested by applying the following mathematical models: the zero-order kinetics model (released drug cumulative percent vs time), the firstorder kinetics model (log cumulative drug percent retained vs time), the Higuchi model (cumulative percent released vs time<sup>1/2</sup>) and Korsmeyer Peppas model (log of released drug cumulative percent vs log time).<sup>14,15</sup>

The kinetic model that best fitted the dissolution data was evaluated by comparing the coefficient of

determination ( $r^2$ ) values obtained in various models. The Peppas model uses the "n" (release exponent) value to characterize different release mechanisms. The values of n = 0.5 for Fickian diffusion, between 0.5 to 1.0 for non-Fickian diffusion and n = 1 for zero order.<sup>16</sup>

### **RESULTS AND DISCUSSION Density evaluation**

Table 1 displays the results obtained for experimental density measurements.

#### Microparticle size

As a result of the correct appreciation of the experimental parameters, we were able to obtain most microparticles ranging between 400 and 630  $\mu$ m, as shown in Fig. 1.

#### X-ray diffractometry

No drug peaks appeared throughout the X-ray diffraction patterns of the gelatin microparticles. The drug was dispersed into the gelatin network while in amorphous state.

Table 1
Density of the tested XN microparticles

$\rho$ (g/cm <sup>3</sup> )
0.821±0.013
$0.832 \pm 0.021$
$0.849 \pm 0.023$
$0.922 \pm 0.027$
0.919±0.019

\*each data point is the average of at least six measurements Table 2

Drug content (g/g of microparticles)

Type of microparticles	W <sub>XN</sub>
(NXN)	$0.1008 \pm 0.0026$
(CXN24)	$0.0973 \pm 0.0039$
(CXN48)	$0.0904 \pm 0.0056$
(CXN72)	$0.0938 \pm 0.0023$
(CXN2SOL)	$0.0557 \pm 0.0097$

\*each data point is the average of at least six measurements

#### **Drug content**

The total drug content of the gelatin microparticles is shown in Table 2.

The effect of the crosslinking agent type was, therefore, evaluated. The drug loading was not affected when the crosslinking process was performed in a glutaraldehyde saturated atmosphere. Crosslinking in an acetone solution of glutaraldehyde (35%) resulted in a decrease of drug loading, as well as of swelling degree and drug release rate. Also, during the crosslinking process in the glutaraldehyde solution, the initially spherical microparticles wrinkled and became irregularly shaped, which made their characterization difficult (Fig. 2).



Figure 1: Values for XN gelatin microparticle diameters (each data point is the average of at least four measurements)



Figure 2: Photographic image of CXN2SOL (left) and CXN24 (right) shapes



 $t = 0 \min$ .  $t = 1 \min$ .  $t = 3 \min$ .  $t = 5 \min$ . Figure 3: Penetration of water during the NXN swelling process (D<sub>0</sub> = 360 µm)

# **Swelling kinetics**

Fig. 3 displays the water front penetration in the noncrosslinked microparticles. Similarly, we have analyzed other types of microparticles that have been subjected to further testing (Table 3). The swelling degree of each developed microparticle decreased along with the exposure time of the crosslinking process, as indicated in equation 4:

$$Q = 1.405 - 0.0022t \tag{4}$$

where: Q = swelling degree, t = time

	Table 3	3	
Values	of swelling kin	etics param	ieters

Type of microparticles	D <sub>0</sub> (μm)	$D_{10}=D_{\infty}$ ( $\mu$ m)	п	$k.10^2$ (min <sup>-n</sup> )	Q
(CXN24)	360	487±21	$0.73 \pm 0.04$	7.5±0.9	1.353±0.058
(CXN48)	360	462±15	$0.65 \pm 0.03$	6.0±0.7	1.283±0.043
(CXN72)	360	449±12	$0.56 \pm 0.01$	4.5±0.3	$1.245 \pm 0.034$

\*each data point is the average of at least six measurements

	Release kinetics parameters				
MCS turno	0 order	1 <sup>st</sup> order	Higuchi	Korsmeyer-	$D \cdot 10^6$
web type	$k \cdot 10^4$	$k \cdot 10^2$	$k \cdot 10^3$	Peppas	$(\text{cm}^2.\text{min}^{-1})$
	$(g.min^{-1})$	$(\min^{-1})$	$(g.min^{-1/2})$	$k \cdot 10^2$	
	2761025	2.81+0.20	260 + 0.15	$3.82 \pm 0.42$	
(NXN)	$2.70\pm0.55$ $r^{2}=0.8807$	$2.81\pm0.20$ $r^{2}-0.0676$	$2.00\pm0.13$ $r^2=0.0026$	$r^2 = 0.9601$	$7.80 \pm 0.33$
	1 -0.8897	889/ 1=0.90/0 1=0.9930	1 -0.9930	$(n=0.465\pm0.034)$	
	$2.02 \pm 0.41$	2 15 1 0 57	1.92+0.20	$1.96 \pm 0.18$	
(CXN24)	$2.03\pm0.41$	$2.15\pm0.57$	$1.83 \pm 0.29$	r <sup>2</sup> =0.9612	$4.32 \pm 0.19$
	r = 0.9629 $r = 0.9457$ $r = 0.9457$	r =0.9916	$(n=0.547\pm0.047)$		
	1.04+0.12	1.26+0.22	0.00 + 0.11	0.76±0.09	
(CXN48)	$1.04\pm0.12$	$1.26\pm0.23$	$0.89\pm0.11$	$r^2 = 0.9816$	$1.70 \pm 0.13$
	r =0.9962	r = 0.9623	r = 0.95/1	$(n=0.942\pm0.064)$	
	0 (1 + 0 - 0 4	0.00 0 1 1	0.52+0.00	0.52±0.06	
(CXN72)	$0.61\pm0.04$	$0.92\pm0.11$	$0.53\pm0.08$	$r^2 = 0.9856$	$1.02 \pm 0.09$
	r =0.99999	r =0.9830	r =0.9456	$(n=1.012\pm0.032)$	

Table 4 Values for the release kinetics parameters ( $D_0 \in 400 - 500 \ \mu m$ )

\*each data point is the average of at least six measurements



Figure 4: A schematic representation of the release mechanism from microparticles (A: the swelling process; B: the drug release)

# Study of XN release from gelatin microparticles

The values of drug release parameters are presented in Table 4.

The drug is released from the obtained microparticles during the initial swelling phase, a process which is on-going after the swelling process ends (Fig. 4A). The XN release is rate-determinant.

As the gelatin microparticles are spherical, we were able to appreciate that the drug release rate follows a spherical symmetry diffusion model (Fig. 4B).

The coefficients for the apparent diffusion (D) have been calculated using the following equation:<sup>18,19</sup>

$$m_{t} = m_{0} \left[ 1 - \frac{6}{\pi^{2}} \sum_{n=1}^{\infty} \frac{1}{n^{2}} \exp\left(-\frac{n^{2} \pi^{2} Dt}{r_{0}^{2}}\right) \right]$$
(5)

where:

 $m_0$  = amount of XN from the microparticles at time 0

 $m_t$  = amount of XN at time t

 $r_0$  = mean microparticle radius

#### **CONCLUSION**

1. An increase in the microparticle density was correlated with an increase in the crosslinking degree.

2. The crosslinking, performed in a 35% solution of glutaraldehyde in acetone, does not only influence the spherical shape of the particles, but also the drug loading. However, the

crosslinking in a saturated atmosphere of glutaraldehyde vapors does not affect these parameters. The XN gelatin microparticles that were crosslinked for 24, 48 or 72 hours in a saturated atmosphere of glutaraldehyde did maintain both their spherical shape and drug loading, providing a robust method for microparticle formulation.

3. Experimental data indicated that the exposure time for the crosslinking process has an impact upon the swelling kinetics of the microparticles, as it also modifies the drug release rate. The crosslinking degree influences the mechanism of water transportation within the microparticles. A higher degree of crosslinking implies a transport mechanism fitting Fick's model, while a lower crosslinking degree translates into a mechanism that becomes abnormal.

4. Drug release from the gelatin microparticles occurs both in the initial swelling phase, as well as after the swelling process ends. Because NXN and CXN24 microparticles have a spherical shape, we appreciated that the drug release follows a spherical symmetry diffusional model. Therefore, a diffusional model for the XN delivery from both types of gelatin microparticles was established (Fig. 4). An increase of the crosslinking degree determines the modification of the kinetic release mechanism from Higuchi to zero order. The process of the XN release is ratedeterminant. Thereby, the values of the kinetic parameters decrease as the crosslinking degree increases.

5. The experimental values of the apparent diffusional coefficients decrease along with an increase in the crosslinking degree.

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