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Research Paper

Characterization and in vivo evaluation of ocular minitablets prepared with different bioadhesive Carbopol–starch components

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Abstract

The purpose of this study was to evaluate different bioadhesive ocular formulations based on drum dried waxy maize® starch (DDWM), Amioca® starch and Carbopol® 974P. The concentrations of Carbopol® 974P in the mixtures varied between 5 and 25% (w/w). The rheological properties of the non-sterilized and gamma-irradiated physical blends of Carbopol® 974P with either DDWM or Amioca® were compared to those of the corresponding co-spray dried Amioca® starch/Carbopol® powders. Higher viscosity or consistency values were measured for sterilized co-spray dried powder mixtures containing an amount of Carbopol® 974P equal or above 15% (w/w) compared to the physical blends.

Sustained release minitablets (Ø 2 mm, 6 mg), consisting of sodium fluorescein as model drug and the bioadhesive powders, were manufactured at a compression force of 1.25 kN. Afterwards, the tablets were sterilized with gamma-irradiation. The amount of Carbopol® in the co-spray dried powder mixtures on the one hand and gamma-irradiation on the other hand had no significant influence on the crushing strength and friability of the minitablets evaluated. However, these two factors affected the in vitro release properties of the minitablets. The slowest release was obtained with tablets containing 25% Carbopol® 974P, which unfortunately possess mucosal irritating properties. By using co-spray dried Amioca® with 15% (w/w) Carbopol® 974P, a slower release can be achieved compared to the physical mixtures of DDWM or Amioca® starch with Carbopol® 974P. Moreover, this ocular formulation is very promising and is preferred, as it did not cause any mucosal irritation and released the model drug for at least 12 h, after application in the fornix.

Keywords: Ocular minitablet; Bioerodible; Carbopol; Starch; In vitro release; In vivo study

1. Introduction

Most common ocular diseases are treated with medication administered locally to the eye. Dosage forms for topical application comprise aqueous or oily drops, ointments, gels and delivery systems. Inserts like Ocusert® and soaked collagen shields or films, placed in the lower fornix or on the cornea for a prolonged period of time, have been presented as alternatives to eye-drops in order to improve bioavailability and efficacy and also better patient compliance. These ophthalmic dosage forms are more effective, requiring less frequent administration, and diminishing the number of additives needed [1–2].

Recently an ocular minitablet (Ø 2 mm, 6 mg) with sustained release properties was developed and optimized. The bioadhesive polymers employed were a physical mixture of drum dried waxy maize® starch (DDWM) and 5% (w/w) Carbopol® 974P. This minitablet was bioerodible, well accepted by humans in a preliminary investigation and showed no mucosal irritation potential. The gelling behavior in the fornix is an advantage since it results in an extended residence time of 8 h at the absorption site [3–5].

The aim of present study is to evaluate new bioadhesive powder mixtures in tablets in order to obtain a longer residence time in the fornix, compared to the tablets containing DDWM with 5% (w/w) Carbopol® 974P, used as reference formulation. Bioadhesive mixtures based on DDWM or Amioca® starch with Carbopol® 974P and the corresponding minitablets were characterized and evaluated. Herewith, the influence of varying amounts of Carbopol® 974P (i.e. from 5 to 25%, w/w) in

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combination with DDWM or Amioca starch® was studied. Furthermore, instead of freeze drying, described by Ameye et al., co-spray drying was investigated as modification technique of the polymer mixtures of Carbopol® 974P with Amioca® starch [6].

As ocular dosage forms must be sterile, gamma-irradiation (25 kGy) of the powders and the minitablets was performed and the influence of gamma-rays on the properties of the polymers and the minitablets was examined.

The rheological behavior of the co-spray dried polymers and the physical blends was compared, while for the minitablets physical properties were measured such as the friability, crushing strength, and in vitro release rate. Since, not all powder mixtures were directly compressable, the effect of dry granulation or an extra precompression step was evaluated. Sodium fluorescein, a frequently used diagnostic agent in ophthalmology was selected, because its tearfilm concentrations can be monitored easily as a function of time. The in vivo release property of the most adequate ocular minitablet obtained in vitro was evaluated in human volunteers.

2. Materials and methods

2.1. Materials

Drum dried waxy maize® starch (DDWM), a pregelatinized starch, was supplied by Eridania Béghin-Say Cerestar (Vilvoorde, Belgium) and Carbopol® 974P by Noveon (Cleveland, Ohio, USA). Amioca® starch and its co-spray dried combination with Carbopol® 974P were received from National Starch and Chemical Company (Bridgewater, NJ, USA). Amioca® starch, a pregelatinized waxy starch too, was prepared by jet cooking, followed by spray drying (National Starch and Chemical Company, Bridgewater, NJ, USA). Sodium stearyl fumarate (Edward Mendell Co. Inc., New York, USA) has been established to be the most suitable glidant to be employed in the bioadhesive formulation [7]. Sodium fluorescein was purchased from Sigma Chemical Co. (St Louis, MO, USA)

An isotonic phosphate buffer solution (pH 7.4) was prepared with 4.030 g/l sodium dihydrogen phosphate dihydrate and 16.252 g/l disodium hydrogen phosphate dihydrate from Merck (Darmstadt, Germany).

2.2. Preparation of minitablets

Table 1 presents the composition of the minitablets used in this study. The powders were firstly homogeneously mixed with a pestle in a mortar, and secondly blended in a laboratory mixer for 10 min (Turbula T2A, Willy A. Bachoffen-WAB, Maschinenfabrik, Basel, Switzerland). Due to the poor flowing properties and the low bulk density of most powder mixtures, it was necessary to prepare granules by slugging in order to obtain minitablets of required quality. Large tablets (Ø 13 mm, 250 mg) were compressed at 0.5 kN using an eccentric tabletting machine Korsch (Type EKO, Berlin, Germany). The tablets were crushed in a mortar and the granules obtained were sieved on a Retsch VE 1000 shaker (Retsch, Haan, Germany), equipped with 45, 90, 250 and 500 μm sieves. The granule fractions $F_{45–250\ \mu m}$ and $F_{90–250\ \mu m}$ were used for, respectively, the physically blended powder and the co-spray dried mixtures. A summary of the powder mixtures and granules chosen used for the manufacturing of ocular minitablets is given in Table 2. The choice of the two granule fractions is based on previously performed experiments, demonstrating that otherwise it would be impossible to manufacture minitablets with the settings available on the tabletting machine.

The powder mixtures PM90dd and PM95dd, employed as reference formulations, and the bioadhesive granules were then compressed into minitablets (6 mg) at a compression force of 1.25 kN, using Korsch tabletting machine, but equipped with four concave punches (Ø 2 mm). Afterwards, gamma-irradiation of the minitablet was performed at room temperature, using a 1.8 MCi activity ^{60}Co source (Gammir-I-Sulzer irradiator unicell, IBA-Mediris, Fleurus, Belgium). The dose rate was set at 1.0 kGy/h and the total radiation dose was 25 kGy [5].

2.3. Physical characterization methods

2.3.1. SEM

The surface structure of the powder mixtures was evaluated by scanning electron microscopy (SEM) (JSM 5600 LV-SEM,

Table 1
Composition of ocular minitablets

	Physical mixture based on DDWM				Physical mixture based on Amioca		Co-Spray dried powder mixture based on Amioca and Carbopol			
	PM 95 dd	PM 90 dd	PM 85 dd	PM 75 dd	PM 95 am	PM 85 am	CS 95	CS 90	CS 85	CS 75
DDWM	92	87	82	72	–	–	–	–	–	–
Amioca	–	–	–	–	92	82	92	87	82	72
Sodium fluorescein	2	2	2	2	2	2	2	2	2	2
Sodium stearyl fumarate	1	1	1	1	1	1	1	1	1	1
Carbopol 974 P	5	10	15	25	5	15	5	10	15	25

CS, Co-spray dried; PM, Physical mixture; dd, drum dried waxy maize starch; am, amioca starch.

Table 2
Powder mixtures and granules, used for the preparation of ocular minitablets

Powder mixtures for direct compression	Granules	
	Granule fraction 45–250 μm	Granule fraction 90–250 μm
PM95 dd-n	PM95 dd	CS95
PM90 dd-n	PM85 dd	CS90
	PM 75 dd	CS85
	PM 95 am	CS75
	PM 85 am	

CS, Co-spray dried; PM, Physical mixture; dd, drum dried waxy maize starch; am, amioca starch; n, native powder mixture.

JEOL,Tokyo, Japan). The powders were coated with platinum, with a sputter coater (Auto Fine Coater, JFC-1300, Jeol, Tokyo, Japan) before scanning electron microscopy was performed.

2.3.2. Rheological characterization

Various dispersions were prepared by addition of the native powders and powder mixtures to an isotonic buffer solution (PBS, pH 7.4). The dispersions were stirred for 1 h at room temperature, on a magnetic stirrer (Thermolyne HP46820-26, Dubuque, IO, USA). Afterwards the dispersions were stored at 6 °C for a least 12 h. The rheological analyses were performed with a controlled stress rheometer (Carri-Med CSL^2 100, TA Instruments, Brussels, Belgium) equipped with a 4 cm acrylic cone for high viscous samples (1.59° acrylic cone, truncation 57 μm) or a double concentric cylinder for low viscous samples. The rheological characteristics were measured at 32.0 ± 0.1 °C, the temperature on the eye surface [8]. A pre-shear procedure was used to homogenize the samples. The test samples were equilibrated for 5 min allowing the polymers to recover from the destruction caused by the pre-shear procedure.

During a flow procedure the shear rate was increased from 0 to 250 s^{-1}. Flow measurements were used to study the relation between the stress (related to the force applied) and the shear rate on the samples, and to determine the viscosity and the flow characteristics [5,9]. Three flow curves were recorded and analyzed using the mathematical Herschel-Bulkley model to calculate the consistency and the shear rate index:

$$\sigma = \sigma_y + K\gamma^n \quad (1)$$

with σ, shear stress (Pa), σ_y, yield stress (Pa), γ, shear rate (1/s), K, consistency index (Pa.s) and n, shear rate index, ranging from $k = 1$ for Newtonian liquids and $k = 0$ for non-Newtonian liquids.

The dynamic viscosity measurements were performed to explain the differences in drug release behavior of the different matrices. Firstly dispersions of native DDWM, Amioca® starch and Carbopol® 974P were evaluated, secondly the preparations of co-spray dried mixtures of Amioca® starch and Carbopol® 974P and finally dispersions containing the physical mixtures of Carbopol® 974P and DDWM or Carbopol® 974P and Amioca® starch.

2.3.3. Crushing strength

An instrumented uniaxial press Lloyd (type L1000R, Lloyd Instruments, Segenworth, Fareham, UK), equipped with a 20 or 500 N load cell was used to analyze the behavior of the non-sterilized and the gamma-irradiated minitablets under force [4]. The data were obtained from 10 tablets, prepared at the same compression force.

2.3.4. Friability

The friability of the minitablets was determined by subjecting 10 tablets weighed together with 100 glass beads (average diameter of 4 mm) to falling shocks for 10 min in an Erweka friabilator (TA3, Offenbach/Main, Germany), set at a speed of 25 revs./min. After 10 min, the glass beads were removed. The tablets were then reweighed and the percentage friability was calculated [4].

2.3.5. In vitro release studies

As dissolution apparatus, vials in an oscillating water bath were employed to evaluate the release of sodium fluorescein from the minitablets ($n = 3$). This dissolution method is the most appropriate to obtain a suitable in vivo simulation [4]. A minitablet was weighed, and transferred to a glass vial containing 1.00 ml isotonic phosphate buffer solution (pH 7.4). To avoid water evaporation, the vials were covered with rubber caps and placed in an oscillating (25 rpm) water bath at 32 ± 1 °C. Aliquots of 80 μl were withdrawn throughout the experiment at 30, 60, 90, 120, 180, 240, 300, 360 and 1440 min, and replaced by an equal volume of fresh buffer solution. The samples were diluted and centrifuged at 4000 rpm for 10 min. The concentration of sodium fluorescein was determined spectrophotometrically using a Perkin–Elmer Lambda 12 UV/Vis (Überlingen, Germany) with Winlab-software (Perkin–Elmer, Überlingen, Germany). The percentage released at each time point was expressed as a fraction of the total amount completely released after 24 h. The profiles were evaluated by the zero and the first order model (k_0, k_1 are the release rate constants), the Hixson–Crowell model (k_{HC} is the dissolution rate calculated from the Hixson–Crowell plot for sink conditions) and the Higuchi model (k_H). Excel 2000 (Microsoft®, Redsmond, WA, USA) was employed for the calculation of the release rate constants (K_x) with the Solver tool and the determination of the correlation coefficients (R).

2.3.6. In vivo study

The in vivo release was studied in six healthy volunteers (three men and three women) with a mean age of 38.6 ± 16.0 year. The basic principles of clinical research formulated in the World Medical Association.

Declaration of Helsinki were taken into account. The concentration of sodium fluorescein after application of a minitablet was measured with a fluorophotometer Fluorotron™ Master (Ocumetrics, Mountain View, CA, USA). Firstly three blank scans were performed for the correction of autofluorescence of the tearfilm-cornea compartment due to the presence of endogenous fluorophores [3,4] Afterwards

a minitablet was positioned temporally in the fornix using a device. The fluorescein concentration in the tearfilm-cornea compartment was measured as a function of time. A wash out period of at least 2 days was applied between each test. The parameters characterizing the in vivo properties of the formulations are $D_{>50\ \mathrm{ng/ml}}$, $D_{>75\ \mathrm{ng/ml}}$ and $D_{>100\ \mathrm{ng/ml}}$. They are defined as the time spans during which the tearfilm-cornea concentrations of sodium fluorescein amount at least 50, 75 and 100 ng/ml, respectively [4]. To test the statistical significance, a one-way analysis of variance (ANOVA) was performed ($P<0.05$).

3. Results and discussion

3.1. Physical characterization of the powders

SEM photographs of the co-spray dried powder mixture containing 15% Carbopol® 974P (CS85) and the physical mixture with 15% Carbopol® 974P based on drum dried waxy maize® (PM85dd) are presented in Fig. 1. The scanning electron microscopy pictures revealed that by spray drying Carbopol® surrounds and is incorporated in the starch granules, while in the physical blends small spheroids of Carbopol® 974 P (∼1–5 μm) are observed, dispersed on the surface of a starch particle.

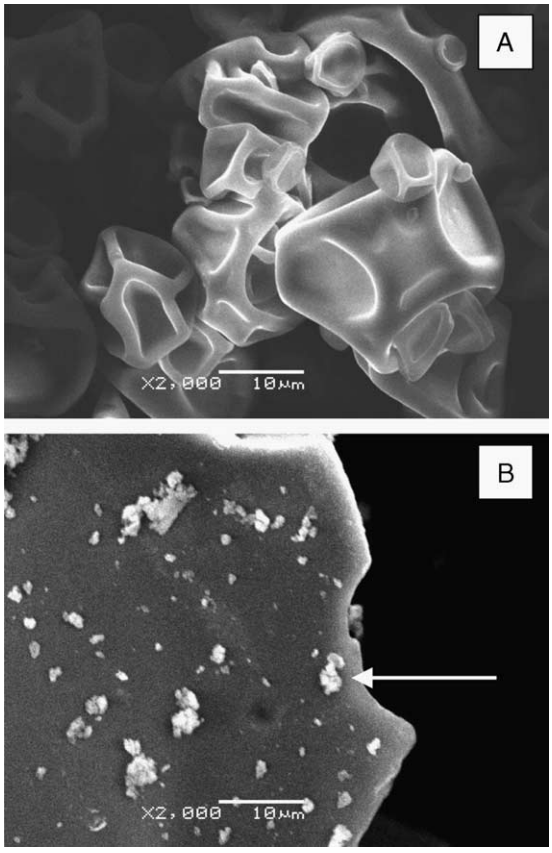

Fig. 1. Scanning electron micrographs of CS85 (A) and PM85dd (B). The arrow points to the Carbopol® 974P particles.

3.2. Rheological characterization of the powders dispersed

The flow results of the rheological characterization of the 30 different dispersions are expressed as consistency, rate index and the viscosity value determined at shear rate 100 s^{-1} (Table 3). The flow rate indices are increased for the dispersions prepared with the gamma-irradiated powder mixtures, compared to the non-sterilized samples. Also lower viscosity and consistency values were measured after dispersing the powders sterilized by gamma-irradiation.

The dispersions of native non- and sterilized polymers were rheologically characterized in order to gain insights in their gel properties and to evaluate the influence of gamma-irradiation on the polymer network structure. The choice of the Carbopol® 974P concentrations 0.8 and 1.9% (w/w) is based on similarity with the Carbopol® 974P concentrations present when dispersing 6% (w/w) of the co-spray dried mixtures or physical blends with 15 or 25% (w/w) Carbopol® 974P, respectively. The results obtained indicated that increasing the concentration of Carbopol® 974P from 0.8% (w/w) to 1.9% (w/w) in the dispersions caused an increase of the consistency and the viscosity values determined at shear rate 100 s^{-1}. It is obvious that at higher polymer concentrations, more resistance occurred against the increase of the stress applied. A Newtonian behavior of the dispersions with gamma-sterilized DDWM or Amioca® starch, can be derived from the rate indices which have a value of almost one. Gamma-irradiation induces a decrease of the gel strength, due to a decrease in amylopectin fraction, which is responsible for the swelling and rheologic properties of starches [10].

The rheological results show that Carbopol® 974P dispersions possess higher consistency and viscosity values and lower flow rate indices than the dispersions prepared with DDWM or Amioca®. Increasing the amount of Carbopol® 974P in the co-spray dried and physical powder mixtures lead to higher viscosity and consistency values and lower rate indices. Consequently, the rheological properties and the pseudoplastic behavior of the polymer mixtures dispersed originate mainly from Carbopol® 974P but not from the starch components. The viscosity and consistency values and pseudoplastic properties are smaller for the dispersions prepared with the co-spray dried mixtures with an amount less than 10% Carbopol® (w/w) compared to those prepared with the physical blends containing DDWM or Amioca® starch.

3.3. Crushing strength and friability

The friability values of all minitablets were below 1.00% (w/w), except for the sterilized minitablets prepared with granules composed of 5% Carbopol® 974P and 89% DDWM (PM95dd) having a friability value of 1.85% (w/w).

The crushing strength of the minitablets is higher when the amount of Carbopol® 974P in the physical powder mixtures (PM95 dd vs. PM95 dd, PM95 dd-n vs. PM90 dd-n and PM95 am vs. PM85 am) is increased. Contrary, the amount of Carbopol® 974P in the co-spray dried powders had no

Table 3
Rate index, consistency and viscosity values (Pa.s) of dispersions prepared from native polymers and polymer mixtures (mean value ± SD, $n=3$)

Sample	C[a]	Viscosity (Pa.s)[b]		Flow rate index		Consistency (Pa.s)	
		0 kGy	25 kGy	0 kGy	25 kGy	0 kGy	25 kGy
Native polymer							
DDMW®	6	0.101 ± 0.003	0.006 ± 0.001	0.699 ± 0.003	1.036 ± 0.008	0.402 ± 0.015	0.005 ± 0.001
Amioca® starch	6	0.103 ± 0.003	0.005 ± 0.001	0.711 ± 0.001	1.042 ± 0.016	0.358 ± 0.010	0.004 ± 0.004
Carbopol® 974P	0.8	0.177 ± 0.003	0.145 ± 0.006	0.502 ± 0.004	0.569 ± 0.006	2.760 ± 0.244	2.105 ± 0.260
	1.9	1.690 ± 0.052	0.554 ± 0.036	0.310 ± 0.007	0.523 ± 0.010	45.010 ± 1.713	12.563 ± 0.707
Co-spray dried polymer mixture (Amioca® starch:Carbopol® 974P)							
95:5	6	0.076 ± 0.001	0.014 ± 0.001	0.803 ± 0.001	0.945 ± 0.007	0.188 ± 0.003	0.018 ± 0.001
90:10	6	0.188 ± 0.002	0.051 ± 0.002	0.695 ± 0.002	0.740 ± 0.005	0.763 ± 0.012	0.156 ± 0.003
85:15	6	2.422 ± 0.008	0.287 ± 0.006	0.334 ± 0.001	0.704 ± 0.007	51.090 ± 0.195	0.723 ± 0.023
75:25	6	11.573 ± 0.667	5.835 ± 0.183	0.287 ± 0.017	0.150 ± 0.066	186.133 ± 13.326	116.90 ± 7.970
Physical polymer mixture (Carbopol® 974P:DDMW®)							
95:5	6	0.168 ± 0.004	0.015 ± 0.000	0.671 ± 0.001	0.929 ± 0.002	0.763 ± 0.016	0.021 ± 0.001
90:10	6	0.312 ± 0.004	0.045 ± 0.001	0.623 ± 0.001	0.814 ± 0.001	1.764 ± 0.027	0.106 ± 0.002
85:15	6	1.033 ± 0.031	0.120 ± 0.005	0.571 ± 0.002	0.707 ± 0.002	7.114 ± 0.220	0.466 ± 0.023
75:25	6	3.380 ± 0.360	0.668 ± 0.010	0.323 ± 0.010	0.613 ± 0.005	75.500 ± 9.574	3.314 ± 0.092
Physical polymer mixture (Carbopol® 974P:Amioca® starch)							
95:5	6	0.375 ± 0.006	0.017 ± 0.001	0.776 ± 0.006	0.907 ± 0.015	1.005 ± 0.035	0.026 ± .003
85:15	6	0.993 ± 0.035	0.134 ± 0.004	0.617 ± 0.012	0.697 ± 0.005	5.324 ± 0.318	0.542 ± 0.033

[a] Concentration (w/w).
[b] Viscosity (Pa.s) at shear rate 100 s^{-1}.

influence on the crushing strength of the minitablets, prepared at 1.25 kN (Fig. 2). Co-spray drying of the powders causes a change in crystallinity and the formation of amorphous material, as a result of a rapid solidification. These structures cause a larger deformability and may lead to a stronger binding between the particles [11].

When comparing the results of tablets prepared with PM95dd and PM95am or PM85dd and PM85am, one can conclude that the spray or drum dried starch types have no influence on the crushing strength of the minitablets. Gamma-irradiation has also no significant influence on the hardness of the minitablets prepared ($P>0.05$). The method of slugging used during preparation of the minitablets, resulted in a decrease of the crushing strength of the tablets, thus minitablets prepared by direct compression led to stronger tablets. This phenomenon is well known as the work hardening principle [12,13]. The loss in compactibility occurs predominantly for plastically deforming materials, such as the starches employed in this study.

3.4. In vitro release studies

The release rate constants K_x calculated with the various mathematical methods proposed are summarized in Table 4. For each of the series examined the most adequate fits are achieved by applying the first order, the Hixson–Crowell and Higuchi equation. A poor fit is obtained, using the zero order model.

The release of drug molecules from a gel is determined (structural organization, diffusion capability and strength of the gel), and by the processes of both polymer swelling and gel layer erosion. Like most unlimited swelling hydrogel matrices, the drug release mechanism is diffusion controlled from the gel forming minitablets [14]. As it can be deduced from the data of the tablets, the in vitro release of the model drug presents mainly a first order kinetic rate.

The reference minitablets (PM95dd-n) prepared by direct compression and the minitablets (PM95dd) have similar average release rate constants (Table 4). Comparing all average release rate constants, the release of sodium fluorescein is faster from the gamma-irradiated minitablets than the corresponding non-sterilized tablets. An explanation, however, cannot be given at the moment for this fast disintegration and release properties of the non-sterilized CS75 minitablet in comparison to the corresponding sterilized CS75 minitablets.

For the minitablets irradiated at 25 kGy, the average release rate values were smaller when the amount of Carbopol® 974P was increased. As proven by the rheological data, PM75dd and CS75 form a strong gel and consequently the release from these sterilized minitablets is slower, (Fig. 3 and Table 4). Adriaens et al. [15] reported however that these two powder mixtures induce a slight irritation on the mucosa [15]. They observed also that the mixing process had only a minor effect on the irritation potency.

Furthermore, a slower release is achieved from the minitablets prepared by using co-spray dried Amioca® with 15% (w/w) Carbopol® 974P (CS85), compared to the physical mixtures of Amioca® starch (PM85a) or drum dried waxy maize starch® (PM85dd) with Carbopol® 974P. Significant differences are obtained from K_{HC} and K_H values of CS85 and PM85dd with respectively P-values of 0.033 and 0.039.

Moreover, the sterilized CS85 minitablet is preferred, since it does not cause any mucosal irritating properties and will be therefore selected for in vivo experiments in healthy volunteers [15].

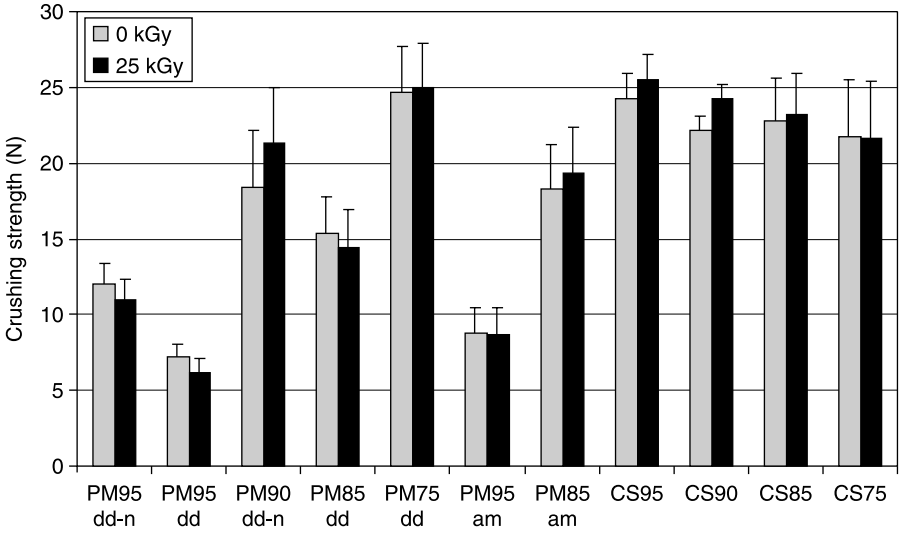

Fig. 2. The crushing strength of the different minitablets prepared [mean (SD)]. CS (Co-spray dried), PM (Physical mixture), dd (drum dried waxy maize starch), am (amioca starch) and n (native powder mixture).

3.5. In vivo study

The in vivo behavior was analyzed of the sterilized CS85 minitablets and the reference formulation (PM95dd-n) prepared with direct compression. Lacrimation occured mostly during a very short time period (about 1 min), directly after application of minitablets in the fornix. After 10–30 min, the volunteers were not aware anymore of the presence of the minitablet applied. The minitablet was completely hydrated, 30 min after application and a viscoelastic gel was formed, determining the release of sodium fluorescein [3,4].

The mean concentration of sodium fluorescein in the tearfilm-cornea compartment after application of the minitablets is presented in Fig. 4. The hydrated tablet sometimes eroded, as a result of high shear forces during blinking and small particles might cause blurred vision during a few seconds. This was mainly observed 4 and 9 h after application of PM95dd-n and CS85 minitablets, respectively. The sterilized minitablets prepared with the co-spray dried mixture Amioca® starch with Carbopol® 974P (CS85) demonstrated a slower release pattern than the reference matrix (PM95dd-n). The residence time of sodium fluorescein in the tearfilm-cornea compartment was also longer, compared to the PM95dd-n preparation.

For the minitablets prepared with PM95dd-n, more than 50 ng fluorescein/ml was measured only up to 6 h, while in the case of CS85 minitablets, the concentration in the tearfilm-cornea compartment remained above 50 ng/ml between 4 and 11 h after their application in the fornix.

The mean parameters $D_{>50\ \mathrm{ng/ml}}$, $D_{>75\ \mathrm{ng/ml}}$ and $D_{>100\ \mathrm{ng/ml}}$ are summarized in Table 5. No significant differences were observed between the two formulations comparing the average $D_{>50\ \mathrm{ng/ml}}$ $D_{>75\ \mathrm{ng/ml}}$ and $D_{>100\ \mathrm{ng/ml}}$ values. High sodium fluorescein levels in the tearfilm-cornea compartment are obtained for a long period of time, after application of the ocular minitablets. The release pattern of sodium fluorescein was influenced by erosion due to the blinking movement of the eyelids and diffusion of sodium fluorescein out of the matrix, as reported in our previous studies [3,4]. An explanation for the slower release pattern

Table 4
Release rate constants (mean ± SD, $n = 3$) calculated after fitting the release profiles

Mathematical model	Release rate constants K_x										
	PM95dd-n	PM95 dd	PM90dd-n	PM85dd	PM75dd	PM95am	PM85am	CS95	CS90	CS85	CS75
First order (10^{-3})											
0 kGy	8.6±2.5	8.0±1.3	7.3±1.3	7.1±2.1	4.0±0.4	9.3±2.3	7.0±0.8	7.2±1.8	6.2±0.8	6.8±1.4	28.6±9.8
25 kGy	17.9±7.0	20.9±6.3	15.1±3.2	12.7±2.0	7.9±0.9	18.3±7.7	13.7±4.7	17.3±2.0	14.4±3.8	9.2±2.8	7.7±1.1
Hixson–Crowell (10^{-3})											
0 kGy	10.1±2.3	9.4±1.5	8.6±1.6	7.9±1.3	5.7±0.3	9.7±1.6	8.0±0.8	8.0±1.3	7.6±1.0	7.8±1.0	24.1±4.0
25 kGy	16.6±0.9	17.9±0.7	12.1±1.2	10.9±1.0	7.6±0.4	16.0±1.5	9.2±1.5	18.0±3.1	11.6±0.8	8.7±0.7	8.0±0.4
Higuchi											
0 kGy	5.3±0.4	5.2±0.3	5.0±0.4	4.8±0.4	4.1±0.2	5.3±0.4	4.9±0.2	4.9±0.3	4.8±0.3	4.8±0.3	8.1±0.4
25 kGy	6.9±0.3	7.1±0.4	6.0±0.3	5.6±0.2	4.8±0.1	6.9±0.5	5.2±0.4	7.1±0.7	6.0±0.2	5.1±0.2	5.0±0.2

CS, Co-spray dried; PM, Physical mixture; dd, drum dried waxy maize starch; am, amioca starch; n, native powder mixture.

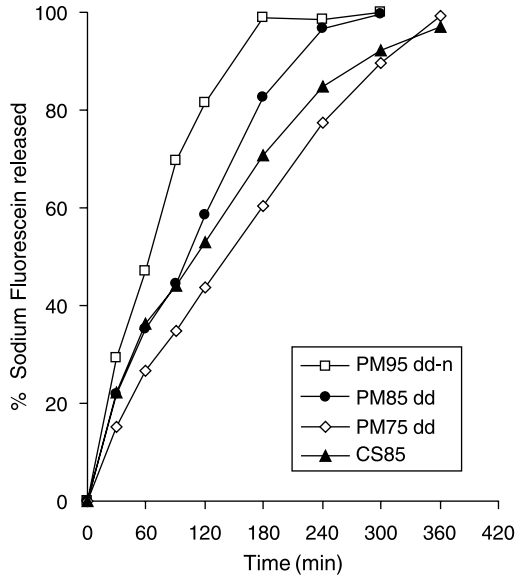

Fig. 3. Sodium fluorescein release profiles of ocular minitablets, sterilized at 25 kGy. CS (Co-spray dried), PM (Physical mixture), dd (drum dried waxy maize starch), and n (native powder mixture).

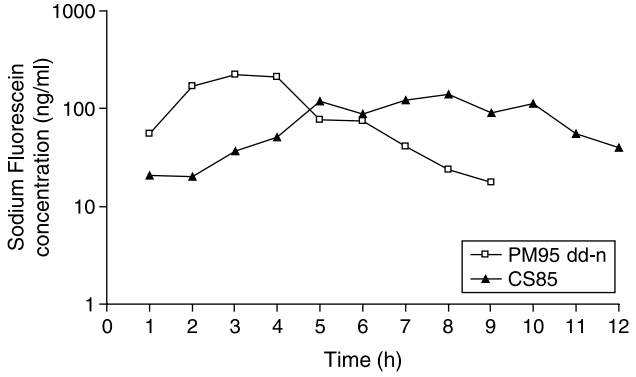

Fig. 4. The tearfilm-cornea compartment concentrations of sodium fluorescein after application of the reference PM95 dd-n and CS85 minitablets, sterilized at 25 kGy.

can be given as follows. First of all, the crushing strength of CS85 minitablets is higher than PM95dd-n preparations, and it has been reported that this property influences the release profile of an insert or minitablet [4,16]. Additionally, from the rheological characterization and in vitro release profiles, one can also derive that the gel strength of the hydrated tablet is higher when CS85 instead of PM95dd-n was used. Consequently, the erosion forces affect less the CS85 gel than the hydrated PM95dd-n tablet, as confirmed by the volunteers who complained about blurred vision. All these characteristics indicated a slower release of fluorescein from CS85 than from PM95dd-n.

Table 5
In vivo results, obtained in six volunteers after application of a minitablet, sterilized at 25 kGy in the fornix (mean ± SD)

Formulation	$D_{>50\ ng/ml}$ (hrs)	$D_{>75\ ng/ml}$ (hrs)	$D_{>100\ ng/ml}$ (hrs)
PM95 dd-n	5.00 ± 1.67	4.00 ± 1.26	2.66 ± 2.16
CS85	6.33 ± 1.21	4.17 ± 1.72	3.16 ± 1.60

Significantly different results ($P < 0.05$).

4. Conclusions

The dispersions of gamma-irradiated co-spray dried powder mixtures with an amount of Carbopol® 974P equal or higher than 15% (w/w), have higher viscosity or consistency values than the equivalent physical mixtures. By using co-spray dried Amioca® with 15% (w/w) Carbopol® 974P (CS85), a slower release can be achieved compared to the physical mixtures of DDWM or Amioca® starch with Carbopol® 974P. Moreover, CS85 is preferred, as it does not cause any mucosal irritating properties and can be considered as a safe bioadhesive carrier, contrary to CS75. The in vivo release of the ocular minitablets is prolonged by employing the co-spray dried powder mixture CS85 instead of PM95dd-n. Further studies investigating physical blends, composed of DDWM or Amioca® with CS85 can be performed to develop a bioadhesive once-a-day delivery system.

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