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GELATINE GELS AND POLYOXYETHYLENE-POLYOXYPROPYLENE GELS: COMPARATIVE STUDY OF THEIR PROPERTIES

Guzmán M., Aberturas M.R., García F., Molpeceres J.

Departamento de Farmacia y Tecnología Farmacéutica.
Facultad de Farmacia. Universidad Alcalá de Henares.
28871 Alcalá de Henares. Spain.

ABSTRACT

Gelatine gels and polyoxyethylene-polyoxypropylene (Pluronic^{R}) F-108 and F-127 gels were prepared at concentrations ranging between 5 and 25 % (W/V), the former by dispersion at 37°C, the later by dispersion at 4°C. The viscosity, the gel-sol transition temperature and the "in vitro" release kinetics of these gels were compared as a first step for the elaboration of parenteral controlled release formulations. Phenolsulphonftaleine (PR) was used as a tracer.

In all cases the viscosity increased with the rise in the concentration of gelatin (20 to 264 cps for 5 to 20 %) or pluronic (260 and 1,520 cps for 20 and 25 % F-108). The gel-sol transition temperature for gelatine gels was directly related to the concentration. On the contrary, for pluronic gels an inverse relation was observed, being the gel-sol transition temperature higher in copolymers with a large percentage of polyoxyethylene groups (30 ± 0.2 °C for 25 % F-108). In both types of gels, a rise in pH and ionic strength decreased the gel-sol transition temperature, whereas PR increase this temperature. The release of the tracer, from the gels to the aqueous medium, showed a zero-order kinetics and the release rates were inversely proportional to the concentration of gelling agent.

INTRODUCTION

When a drug is administered in a conventional dosage form, does not provide stable therapeutic blood levels for extended time periods; nevertheless , it is usually desirable to maintain a constant drug concentration within the therapeutically effective

concentration range. The use of high viscosity hydromiscible vehicles such as hydrophilic gels is one of the different approaches for controlled drug delivery.

Pluronics[R], non-ionic surface active agents, are block copolymers consisting of polyoxyethylene-polyoxypropylene-polyoxyethylene units. At low concentrations (10^{-4} - 10^{-5} %) they form monomolecular micelles, but at higher concentrations multimolecular aggregates with hydrophobic central core are formed, while their hydrophilic polyoxyethylene chains face the external medium. Their relatively low toxicity and capacity to form clear gels make them particularly suitable for pharmaceutical applications, such as dermatological [1,2] or ophthalmic [3,4] formulations as well as in the area of controlled drug delivery systems [5,6].

Gelatin is a mixture of water-soluble proteins of high molecular weight, derived from collagen. It is able to absorb 5-10 times its weight in water to form a gel in solutions at 35-40°C [7], that has been classically used for sustained-release formulations.

In the present paper, physicochemical characteristics as the viscosity, the gel-sol transition temperature and the "in vitro" drug release kinetics of gelatin and polyoxyethylene-polyoxypropylene gels were compared as a first step for the elaboration of parenteral controlled release formulations. The tracer chosen for this study, phenol red (PR), has been classically used to evaluate renal function "in vivo". Furthermore, due to its spectrophotometric characteristics it is easily quantified.

MATERIALS AND METHODS

Pluronics[R] were a gift from BASF Española S.A. and used as received. Phenol red was purchased from Hopkin and Williams (U.K.) and gelatin (Pharm. Helv. VI) was obtained from Merck. All other chemicals were of reagent grade.

- **Phenol red solution**(Phenolsulphonphthaleine injection) was prepared following the USP XX procedure [8] at a concentration of 5 mg/mL.
- **Gelatin/PR and pluronic/PR gels.** Appropriate amount of gelatin or pluronic were added to the previously prepared PR solution. Gelatin was left overnight at 37°C to complete dissolution, at concentrations ranging between 5% and 20%. Pluronic F-108 20% and 25% and pluronic F-127 20% gels were prepared by Schmolka's cold method[9]
- **Viscosity measurements** were carried out at 37° C with a rotating cylinder synchroelectric viscosimeter (Brookfield RVTD).
- **Gel-sol transition temperature** was determined using the Vadnere et al. technique [10] adapted to our experimental conditions. Briefly, 2 mL of the gel in fluid state were placed in a thermostated glass tube (10 cm length x 0.8 cm i.d.). The temperature was raised to 35°C for Pluronic dispersions or lowered to 20°C for gelatin dispersions and kept

constant for 30 minutes to achieve gel formation. The system was then inverted and the temperature slowly modified at a rate of 1°C per 15 minutes (or 0.1°C in the region of the gel-sol transition temperature) until the gel started to flow. That temperature was assumed to be the gel-sol transition temperature. The effect of solutes, sodium hydroxide (0.1680 g/L) or sodium chloride (9 g/L), on the transition temperature were also studied.

- **"In vitro" release rates studies** were carried out using a thermostated dialysis cell at 37°C. Sample (5 mL) was placed in the donor compartment which was separated by a Visking 2-18/32 inch membrane (Medicell Int. Ltd.) with a surface area of 3.8 cm^2 from the receptor compartment consisting in 100 mL of pH 7.2 phosphate-buffered saline (PBS). PR concentration was analyzed spectrophotometrically (Spectronic 2000, Bausch & Lomb) at 558 nm wavelength in an alkaline medium.

RESULTS AND DISCUSSION

- **Gel-sol transition temperature.** In all cases (Figure 1), it was lower than 37°C and a direct relation between gelatin concentration and transition temperature was observed. The generally accepted model for the gelatin molecule is the triple helix, consisting of three associated left-handed helices. This structure is stabilized by hydrogen bonds between the NH groups on the backbone of one chain and carbonyl groups of a neighboring chain and water as an intermediary in inter and intrachain hydrogen bonding. When heated in aqueous solutions, gelatin will denature and the formation of random-coil gelatin is observed. When the aqueous dispersion of gelatin is rechilled, the gelatin structure re-forms depending on solvent conditions, temperature and concentration [11]. So, the gel-sol transition temperature increase from 28.9 °C to 32.3°C when gelatine concentration was 5 or 20 % respectively. Table 1 summarize the results obtained.

No gel formation was observed at concentrations below 25% for pluronic F-108 and 20% for pluronic F-127. These results do not agree with previous studies in which the minimum concentration needed for gel formation was 20% (W/V) for pluronic F-108 at 37°C [12]. Pluronic gels exhibit a reverse thermal gelation behavior which can be explained as a desolvation and swelling process of the copolymer to form cross-linked aggregates. In aqueous solution, pluronic molecules are surrounded by an hydration layer at low temperatures. But, when the temperature is raised the hydrophilic chains of the copolymer are desolvated due to the breakage of the hydrogen bonds that had been established between the solvent and these chains. This phenomenon favor the hydrophobic interactions among the polyoxypropylene domains, and leads to gel formation.Because of the dehydration process the hydroxyl groups become more accessible and can thus develop intermolecular hydrogen bonds, thereby favoring gel formation [13,14]. When

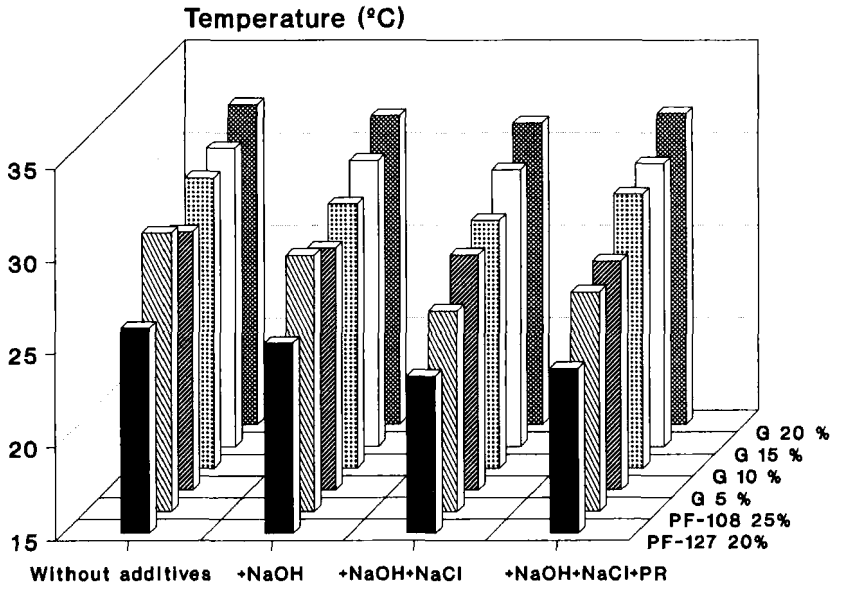

FIGURE 1

Gel-sol transition temperatures for gelatin and Pluronics gels. Each value represents the mean of three experiments.

TABLE 1

Gel-sol transition temperature for Gelatine and Pluronic gels (n=3, mean ± S.D.).

	WITHOUT ADDITIVES	WITH NaOH	WITH NaOH+NaCl	WITH NaOH+NaCl+PR
G 5 %	28.9±0.3	28.0±0.1	27.6±0.1	27.3±0.1
G 10 %	30.6±0.2	29.2±0.1	28.3±0.1	29.7±0.1
G 15 %	31.1±0.1	30.4±0.1	29.9±0.1	30.2±0.1
G 20 %	32.3±0.2	31.7±0.2	31.3±0.1	31.8±0.1
PF108 25%	30.0±0.2	28.8±0.2	25.8±0.2	26.8±0.2
PF127 20%	26.0±0.1	25.2±0.2	23.4±0.3	23.8±0.1

increasing the pluronic concentration, the intermicellar distance and the degree of micellar swelling necessary for polymers to interact are reduced. So, the higher the pluronic concentration the lower the gelation temperature.

The preparation of PR solutions according to the USP procedure, requires the use of NaOH as solubilizing agent and NaCl as isotonizing agent. On account of that, some additives, mainly ionic solutes, can altere the physico-chemical characteristics of these gels. So, we have evaluated the effect of NaOH and NaCl on the gel-sol transition temperature. All additives produced a decrease in the gelation temperature of the gelatin gels. Otherwise, the addition of PR induced a slight increase in gelation temperature compared to that of NaOH plus NaCl formulation. These results suggest that electrostatic interactions may occur between the ionic groups of the gelatin chains and the electrolytes with an opposite charge. When this happens, chain reversals appear and H-bonding formation, and triple helical structure refolding are restricted. In this cases the gelation temperature was lower than for gelatin alone.

The effects of the solutes on the Pluronic gel-sol transition temperature were similar, and the results obtained are agree with those of earlier investigators for pluronic F-127 [6,15], suggesting that when the ionic strength of the solution is increased, part of the water will be engaged in ionic solvation. The energy needed to achieve a given level of polymer desolvation will be reduced, consequently reducing the gel-sol transition temperature. Contrariwise, PR is freely soluble in alkali hydroxide solutions because of sodium salt formation. Since part of these sodium ions are involved in this process, the gel-sol transition temperature increases slightly.

- Concerning **viscosity measurements**, both types of gels showed a direct relation between viscosity and gelatin or pluronic concentrations. However, gelatin dispersions displayed lower viscosities than pluronic ones at 37°C due to its fluid state (figure 2). Gelatin dispersions exhibit a gradual increase of viscosities until a 15% (W/V) concentration is reached, but above 20% (W/V) concentration the interactions between the gelatin molecules are stronger, which explains the higher shear strength. The viscosity of 20% (W/V) pluronic F-108 dispersions was similar to that of gelatin at the same concentration, but 25% (W/V) pluronic F-108 and 20% (W/V) pluronic F-127 showed a dramatic increase in viscosity suitable to their gel state at 37°C.

- **"In vitro" release rates** for PR from gelatin and pluronic gels, to an aqueous medium, is by a zero-order kinetics as shown in figure 3, which expresses the amount of tracer delivered from the gels assayed at 37°C as a function of time. The release rates were determined from the slope of the linear curves obtained from the fitted data. These results as well as the amounts released at 6.5 h and the percentage of PR in the aqueous

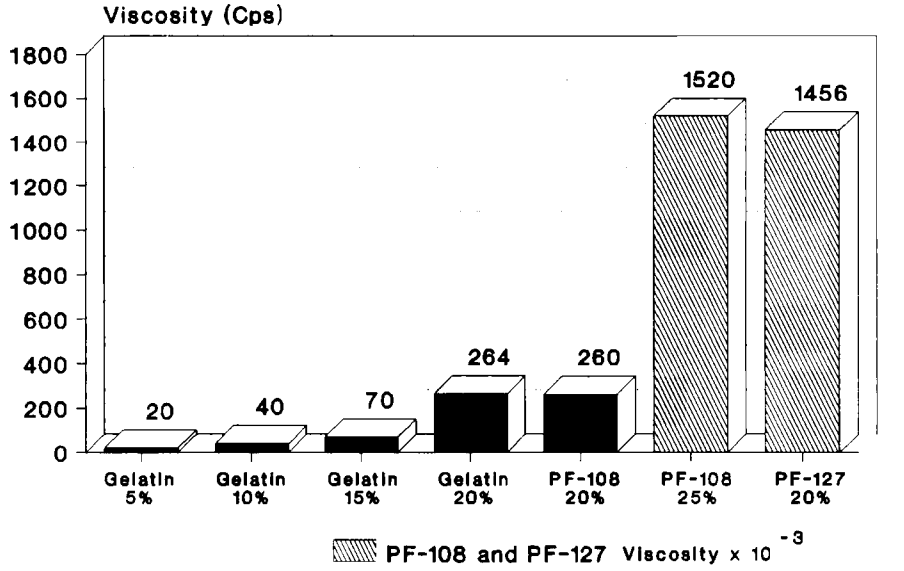

FIGURE 2

Viscosity measurements in centipoises (cps) for gelatin and pluronic gels at 37°C. Each value represents the mean of three experiments.

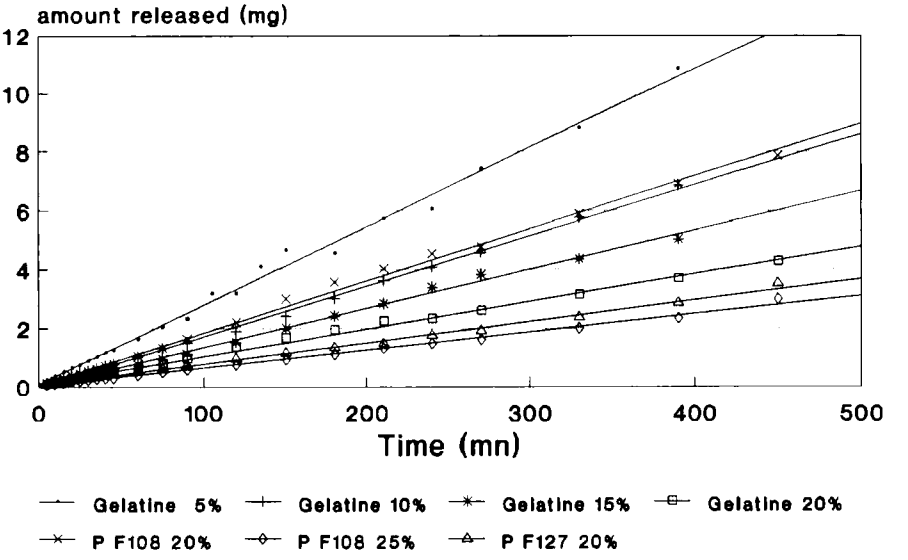

FIGURE 3

Release of PR from gelatin and pluronic gels at 37°C. The concentration of PR was 5 mg/ml. Individual values are the mean of three experiments.

TABLE 2

"In vitro " release rate, amount and percentage of PR released at 6,5 h, from 5 mg/ml gelatin/PR and pluronic/PR gels.

Sample	Release rate (μg/h)	Amount released (mg)	Percentage released
G 5%	26.90	10.88	43.52
G 10%	17.22	6.82	27.56
G 15%	13.32	5.05	20.21
G 20%	9.43	3.70	14.83
F108 20%	17.78	6.92	27.68
F108 25%	6.19	2.35	9.40
F127 20%	7.26	2.86	11.45

medium, referred to the total amount in the gel, are summarized in Table 2. These data support the results obtained for viscosity and transition temperature measurements. The size of the pores created between the crosslinks limits the release rate of the drugs that diffuse through the water channels in the gel matrix [15]. As the concentration of gelatin or pluronic increased and the rigidity of the gels rose, the release rate decreased.

CONCLUSION

Pluronic and gelatin gels showed opposite behavior versus temperature. Pluronic gels are on the liquid state below physiological temperatures, thus allowing their administration by the subcutaneous route. At the administration site, Pluronics are able to form a gel depot acting as a controlled drug release system. Further research about this subject is actually taking place in our laboratory.

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