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Effect of Gamma Irradiation on Pluronic Gels for Ocular Delivery of Ciprofloxacin: in Vitro Evaluation

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Abstract: Gamma irradiation was tested as a method for sterilization of pluronic F-127 gel to be used for ocular delivery of ciprofloxacin (CFX). The effect of gamma irradiation on the properties of pluronic gels was studied at different irradiation doses and pluronic copolymer concentrations. The flow of pluronic gels of different concentrations was non-Newtonian with thixotropic pseudoplastic properties for both non-irradiated and irradiated gels. The viscosities of the irradiated gels increased equally at all doses of irradiation. Deviation from Newtonian flow and degree of pseudoplasticity at different irradiation doses were compared by determination of Farrow's constant values. The values decreased with increasing of irradiation dose indicating a decrease in the degree of pseudoplasticity while non-Newtonian flow was retained at all tested doses. CFX was unstable in pluronic gels irradiated at high irradiation doses for all pluronic concentrations. However, drug instability due to irradiation was less severe as concentration of pluronic was increased indicating more drug protection as the concentration of copolymer increased. At pluronic concentration of 25% w/v, low irradiation doses namely 15 and 20 kGy did not harm the drug and its concentration in the gel was kept within 98 ± 1.59%. Sterility test on pluronic gel (25% w/v) revealed that irradiation dose at 20 kGy can give a definite sterile product. Release study of CFX from the pluronic gel (25% w/v) showed a significant decrease in drug release after 180 min from irradiated (20 kGy) pluronic gel. The developed formulation could be a viable alternative for the ocular delivery of ciprofloxacin by virtue of its desirable properties.

Key words: Pluronic gels, Ciprofloxacin, Gamma irradiation, Rheological properties, Stability, Sterility; Release study.

INTRODUCTION

One of the major problems encountered with the topical delivery of ophthalmic drugs is the rapid and extensive percorneal loss caused by drainage and high tear fluid turnover (Lang, 1995). Typically; ophthalmic bioavailability of only 1-10% are achieved due to the short percorneal residence times of ophthalmic solutions. Treatment compliance can, therefore, be insufficient due to the high frequency of administration (Edsman *et al.,* 1996; Séchoy *et al.,* 2000). Consequently, there is a need for frequent instillation of concentrated solutions to achieve the desired therapeutic affect or to increase the residence time of the drug in the eye by using suitable ocular formulations.

Formulators usually have to design a dosage form which provides a balance between corneal penetration, ocular irritation and formulation stability. Manipulation of formulation parameters to enhance the corneal penetration is one of the approaches of increasing ocular availability (Malhotra and Majumdar, 2001; Ahuja *et al.,* 2008).

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Various ophthalmic vehicles, such as suspensions, ointments, inserts, and aqueous gels, have been investigated to extend the ocular residence time of medications for topical application to the eye (Ranade and Hollinger, 1996). These ocular drug delivery systems offer some improvements over conventional liquid dosage forms but because of blurred vision (e.g., ointments) or lack of patient compliance (e.g., inserts) they have not been universally accepted. As a result, good ocular bioavailability following topical delivery of a drug to the eye remains a challenge yet to be resolved satisfactorily.

An important class of gel-forming polymers that have been widely evaluated as semisolid vehicles for ophthalmic use is poloxamers (Pluronics). Poloxamers, copolymers of poly(oxyethylene)-poly(oxypropylene)-poly(oxyethylene), possesses several properties, such as low toxicity and optical clarity, which render them suitable for use in the formulation of ophthalmic dosage forms. Poloxmers gel was evaluated as ophthalmic drug delivery alone and in combination with other polymers (Miller and Donovan, 1982; Lin and Sung 2000). Pluronics exhibit thermoreversible gelation with increase in viscosity upon increasing temperature. However, they require high concentrations of polymers to form stiff gel upon instillation in the eye (Lin and Sung, 2000; El-Kamel, 2002).

The relationship between the contact time and the rheology is easily understood for viscosity enhanced ophthalmic solutions. During blinking the shearing force on the preparation is large. If the viscosity at high shear rate is too high this will result in irritation. If, on the other hand, the viscosity is too low it will give rise to increased drainage. To decrease the drainage between blinks, the viscosity at low shear should be high. The reheloigcal properties of a gel are most likely of importance for ocular contact time (Edsman et al., 1996).

As the ophthalmic preparations should be sterilized, sterilization process should be carefully selected to be suitable for maintaining the physical and chemical properties of the final ophthalmic dosage form. Radiation offers several advantages as a sterilization method that makes it attractive in growing number of situations. Several research papers described sterilization by radiation, which include a large variety of disposable medical products, sutures and implants, pharmaceuticals, cosmetics, and biological tissues (Swinwood and Wilson, 1990; Dorpema, 1990).

Radiation sterilization technologies and their applications in the manufacture of pharmaceuticals are not well understood by the pharmaceutical industry. Research carried out focused on the treatment of pharmaceuticals with high doses of radiation often resulted in unacceptable colour, odour, and viscosity changes, as well as, undesirable physical and chemical changes (Gopal *et al.,* 1988). It is necessary to analyze each compound for changes in biological activity or for induction of harmful products in order to determine the feasibility of its being exposed to radiation.

Cobalt-60 is one of the most useful gamma sources for radiation processing because of its availability, high gamma energy (1.88 kGy and 2.13 kGy), long half-life (5.27-years), and great penetration capacity (Bruck and Mueller, 1988). Irradiation by cobalt-60 rays is the simplest and most reliable method of sterilization for most medical products and equipment (Bogl, 1985; Gopal *et al.,* 1988; Bruck and Mueller, 1988; Reid and Wilson, 1993).

Radiation treatment can be applied to pharmaceuticals and related products at two levels: raw materials can be irradiated and then aseptically processed into final products, or finished products can be irradiated at the final stage of the manufacturing process. The advantage of the latter approach is its simplicity as a terminal treatment, yielding a product of the desired sterility assurance level. However, it should be recognized that a number of preparations might undergo radiation physical and chemical changes (Bogl, 1985).

In this study, the effect of ionizing gamma radiation in different doses on the physicochemical properties of pluronic F-127 gels containing CFX, as a drug model, was investigated including rheological behaviour of the polymer aqueous solutions, drug stability and release profiles.

MATERIALS AND METHODS

Chemicals:

Ciprofloxacin (CFX) and Pluronic F-127 were obtained from Fluka Chemika, (Switzerland). Acebutolol (internal standard) was obtained from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Solvents used for chromatographic determinations were HPLC grade. All other reagents and solvents were of analytical grade.

Gel Preparation:

Gel formulations containing 0.3 g% of CFX in different pluronic F-127 solutions were prepared. Ciprofloxacin (0.3 g) was dissolved in acetate buffer (pH 4.0) in a 100 ml volumetric flask and the required amount of pluronic was then added to the drug solution and allowed to dissolve with continuous stirring. The formulation was then brought to volume with buffer and thoroughly mixed to get final formulation of concentrations 10, 15 and 25% w/v pluronic. The prepared gel solutions were kept in a refrigerator until use.

Irradiation of CFX Gel:

The prepared CFX gels were subjected to five different doses of gamma radiation (0, 15, 25, 50, 75 and 100 kGy) from Cobalt-60 source in a Gammacell-220 (Nordion International Inc., Kanata, Canada) at a rate of 1.15 Gray/second.

Rheological Behavior of Gels:

The flow properties and changes in rheological behavior of the formulated gel solutions prior and after gamma irradiation were investigated by determining gel viscosity using Brookfield DV-II model RV Viscometer (USA) at different rate of shear. The measurement was made over range of speed from 0.5-20 rpm with 1 minute between each successive speed and in a descending order.

Assay of CFX:

A modified HPLC method was used for determination of CFX in gel formulations. A simple, sensitive and stability-indicating HPLC method with fluorescence detection was developed to determine CFX in gel formulations using modified literature methods (Basci et al., 1996; Thoppil and Amin, 2000). Briefly, the HPLC system consisted of a Waters Model 1515 HPLC pump, a Waters autosampler Model 717 plus (Waters Inc., Bedford, MA, USA), a Waters M-600E fluorescence detector (Waters Inc., Bedford, MA, USA) governed by a microcomputer running Millennium® version 32 software, and vortex mixer (Scientific Industries, Inc., NY, USA). Separation of CFX was carried out with acebutolol as internal standard and a mobile phase consisting of methanol-acetonitrile-acetic acid (5%) (6:7:87, v/v/v) at a flow rate of 1.2 ml min^{-1} at ambient temperature through a Symmetry C_{18} stainless steel column (150 mm length x 3.9 mm i.d, 5 µm particle size). Column effluent was monitored with fluorescence detection at 280 nm (excitation) and 455 nm (emission) at attenuation 2 and gain of 10. Aliquots were loaded in the autosampler tray and volumes of 20 µl were injected into the chromatographic system.

Stability of CFX:

Ciprofloxacin in formulated gels was tested for its stability after gamma irradiation at different irradiation doses using the above HPLC assay method. The most stable formulations were then subjected to sterility test.

Sterility of Gels:

The fluid thioglycollate medium and its alternative method (soybean-casein digest medium) were applied according to the USP guidelines for sterility testing to determine if the prepared samples comply with the requirements set forth in the individual monograph with respect to the test for sterility. Pluronic gels at concentration of 25% w/v were subjected to sterility test after irradiation at different irradiation doses using USP XXIV Method II sterility testing. For microbial contamination test fluid thioglycolate medium was used, while Soybean-casein digest medium was used to test for fungi with incubation under aerobic conditions.

In vitro Release Study:

The release of CFX from the selected irradiated sterile pluronic formulation (25% w/v) was determined using Franz diffusion cell. Cellophane membrane (3.14 cm^2 exposed surface area) was mounted on the receptor compartment. The receptor compartment was filled with 15.0 ml of pH 4.0 acetate buffer maintained at 37± 0.5 ºC and stirred by a magnetic bar at 600 rpm. One gram of gel formulation was placed on the membrane and the top of the diffusion cell was covered with paraffin paper. At appropriate time intervals (30, 60, 90, 120, 180, 240, and 300 min), 3 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh receptor solution to maintain sink conditions. The samples were analyzed for its content of CFX using the HPLC method, as previously described.

Statistical Analysis:

The significance of the differences between releases data were evaluated using Student's t-test. Probable value (p) less than 0.05 were taken as the criterion for statistically significant difference.

RESULTS AND DISCUSSION

Rheological Properties of the Prepared Gels:

The rheological gel properties are considered as one of the crucial factors that should be considered when designing an ocular delivery preparation with emphasize on the ocular contact time. Thus, the evaluation of

eye gels should consider gel viscosity and its changes that may occur upon formulation of the final product. Figures 1-3 showed the flow properties and viscosity of CPX pluronic gels at different gamma irradiation doses for different pluronic concentrations. It was clear that the flow is non-Newtonian with thixotropic pseudoplastic properties. It was also noticed that at all the irradiation doses, the thixotropic pseudoplastic property was retained with some changes in the viscosity upon irradiation. An increase in viscosity of the gels was noticed at low dose of irradiation and same change was noticed even for higher doses [Figures 1-3]. Table 1 shows the effect of irradiation dose on the viscosity of pluronic gels. It has been reported that biodegradable polymers may undergo chain scission and crosslinking after exposure to gamma irradiation (Calis *et al.,* 2002). The observed increase of viscosity of pluronic upon exposure to irradiation could be mainly due to predominant crosslinking effect.

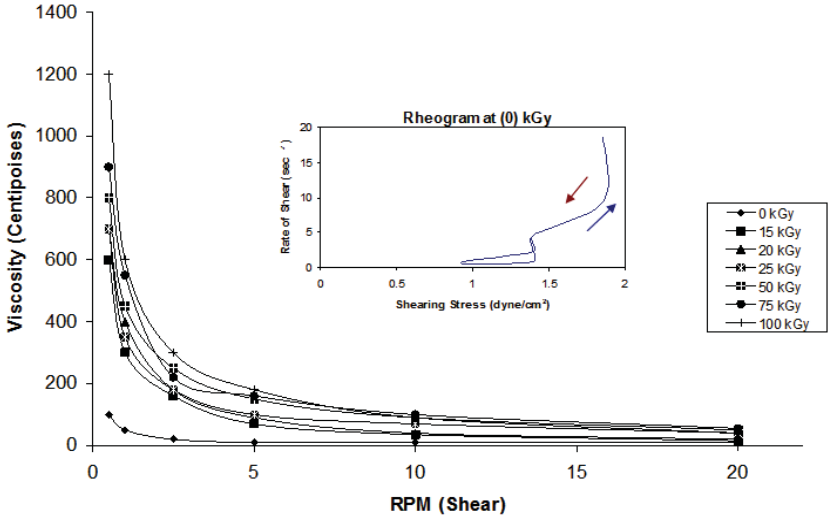

Fig. 1: Effect of irradiation doses on rheological properties of ciprofloxacin pluronic gel (10% w/v).

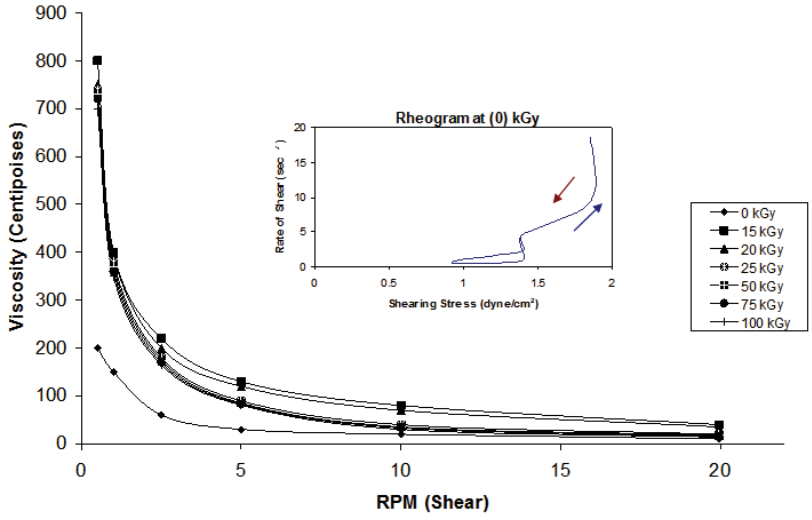

Fig. 2: Effect of irradiation doses on rheological properties of ciprofloxacin pluronic gel (15% w/v).

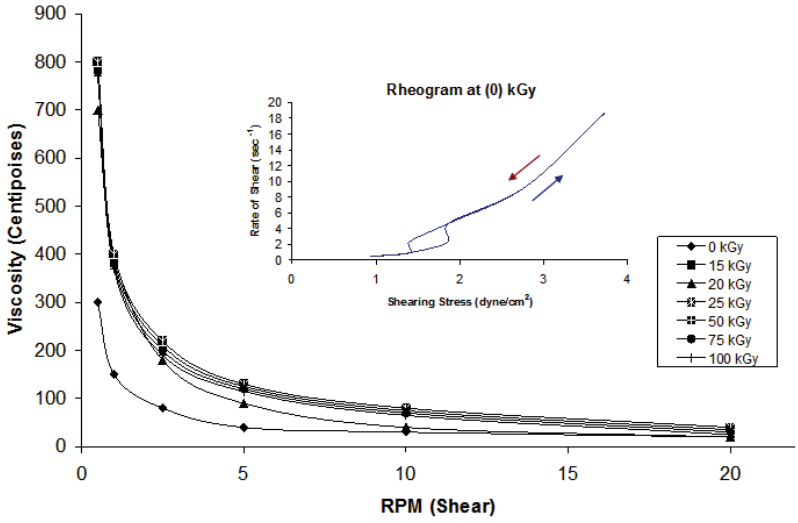

Fig. 3: Effect of irradiation doses on rheological properties of ciprofloxacin pluronic gel (25% w/v).

Table 1: Effect of radiation dose on Farrow's constants (N) of ciprofloxacin gels prepared with the investigated polymers

Formula	Irradiation Dose(kGy)						
	0	15	20	25	50	75	100
Pluronic (10% w/v)	4.70	4.00	3.45	3.19	3.50	3.19	3.80
Pluronic (15% w/v)	4.91	5.25	3.12	2.38	2.52	2.31	2.52
Pluronic (25% w/v)	4.78	3.33	3.23	2.89	2.52	2.02	1.12

The change of viscosity of the irradiated gel was also accompanied by a change in the non-Newtonian behavior of the gels. The non-Newtonian behavior for gamma irradiated gels at different irradiation doses were compared by applying exponential equation (Eq.1) which can be used to differentiate between flow properties of different non-Newtonian systems (El laithy *et al.,* 2002, Ibrahim, 2009).

$$F^N = \eta' G \qquad \text{(Eq. 1)}$$

Eq. 1 could be written in logarithmic form into Eq. 2:

$$\text{Log } G = N \log F - \log \eta' \qquad \text{(Eq. 2)}$$

A plot of log G versus log F gives a straight line which allows for the determination of N value.

Where F is shearing stress, G is rate of shear, η' is viscosity coefficient and N is exponent. The exponent N (Farrow's constant) rises as the flow becomes increasingly non-Newtonian and decreases as the flow becomes decreasingly non-Newtonian. Table 1 shows Farrow's constant (N values) for irradiated gels; it was noticed that as the irradiation dose increased the N value gradually decreased but still exceeding the value of one even at 100 kGy dose. Accordingly, at all doses of irradiation and all concentrations of pluronic gels the flow properties are retained non-Newtonian. The retained pseudoplastic non-Newtonian property upon irradiation is an important property that is required for successfully formulated eye gel. The obtained N values also indicated a fast decrease in non–Newtonian properties for pluronic 25% w/v.

Stability of CFX Post-gamma Irradiation of Gels;

Stability of drug in the formulated gel is an essential parameter that should be considered upon exposure to gamma irradiation and for the determination of the required irradiation dose. Figure 4 showed that CFX was unstable in pluronic gels irradiated at high irradiation doses; this was observed for all pluronic concentrations. However, drug instability was less severe as the concentration of pluronic was increased and it can be concluded that as the concentration of pluronic copolymer increased more protection against effect of gamma irradiation is expected. At pluronic concentration of 25% w/v, low irradiation doses namely 15 and 20 kGy

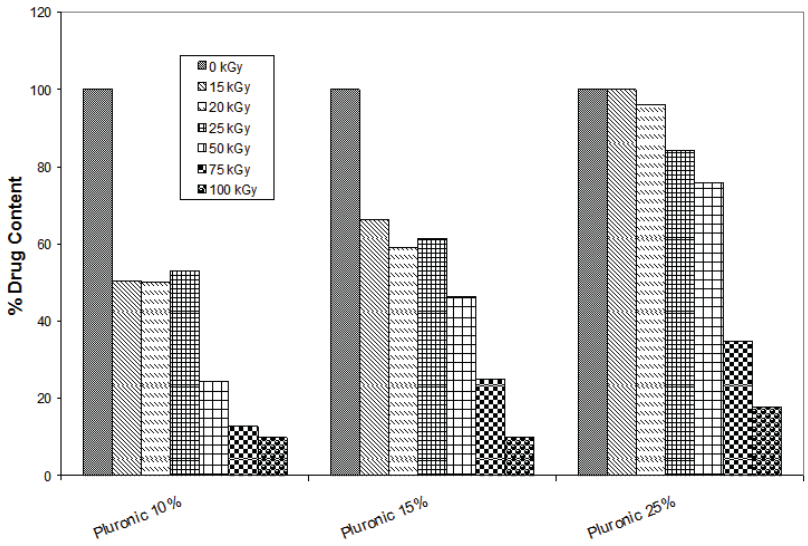

Fig. 4: Percent ciprofloaxcin content in irradiated gel at different radiation dose (Initial drug concentration = 0.3 g %).

did not harm the drug and drug concentration in the gel was kept within 98 ± 1.59 %. Stability of drug in the formulated gel is an essential parameter that should be considered upon exposure to gamma irradiation and for the determination of the required irradiation dose. Figure 4 showed that CFX was unstable in pluronic gels irradiated at high irradiation doses; this was observed for all pluronic concentrations. However, drug instability was less severe as the concentration of pluronic was increased and it can be concluded that as the concentration of pluronic copolymer increased more protection against the effect of gamma irradiation is expected. At pluronic concentration of 25% w/v, low irradiation doses namely 15 and 20 kGy did not harm the drug and drug concentration in the gel was kept within 98 ± 1.59 %. This could be due the presence of drug molecules in the formed polymeric micelles of pluronic F-127 (Guzman *et al.,* 1994; Escobar-Chavez *et al.,* 2006) which leads to drug protection against the low doses of gamma irradiation and/or the high viscosity of the irradiated gel which may also protect to some extent the possible free drug that may be not incorporated in the micellar system. Ciprofloxacin shows partitioning from aqueous to micellar phase in both acidic as well as basic environment (Khan *et al.,* 2009).

Sterility of Formulated Pluronic Gels:

Gamma radiation was chosen as a method for sterilization of the eye gel. The choice of a suitable irradiation dose should depend on the dose that do not harm the drug or affect the physical and chemical properties of the gel, and it should give the required product sterility. Sterility test was performed on pluronic gel of concentration 25% w/v, irradiated at doses of 15 and 20 kGy with CFX concentration of 0.3% w/v. It was observed [Figure 5] that irradiation dose at 20 kGy gave a definite sterile product as indicated from sterility testing for both bacterial [Figure 5A] and fungal contaminations [Figure 5B]. Thus, 25% w/v pluronic gel containing CFX of concentration 0.3% w/v and irradiated at 20 kGy that gave stable sterile product was chosen for the release study of CFX.

Release Study:

Release study was performed to investigate the effect of gamma irradiation upon the release of CFX from the formulated pluronic gels. The study revealed no significant difference in initial CFX release from irradiated (irradiation dose 20 kGy) and un-irradiated pluronic gel of concentration 25% w/v up to about 180 minutes [Figure 6]. This was followed by a significant decrease in drug release from irradiated gel. Initial release could be attributed to the surface drug release with similar profiles for both samples. This was followed by drug diffusion from the formulated gel. The delay in drug diffusion could be due to increase in gel viscosity upon irradiation that was noticed in this study.

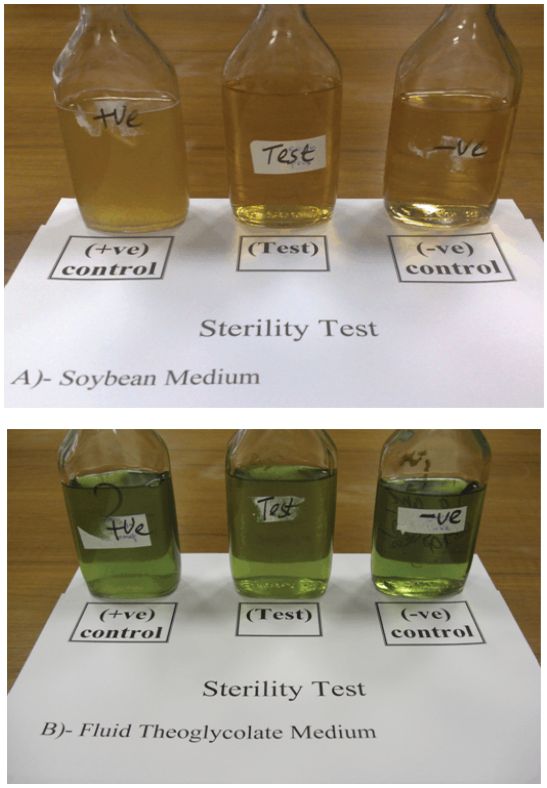

Fig. 5: Sterility testing of pluronic gel of concentration 25% w/v, irradiated at doses of 15 and 20 kGy with CFX concentration of 0.3% w/v for both bacterial (a) and fungal contaminations (B). Positive control: Staphylococcus aureus (bacterial contamination) and *Candida albicans* (fungal contamination), test: inoculated media and negative control: sterile media.

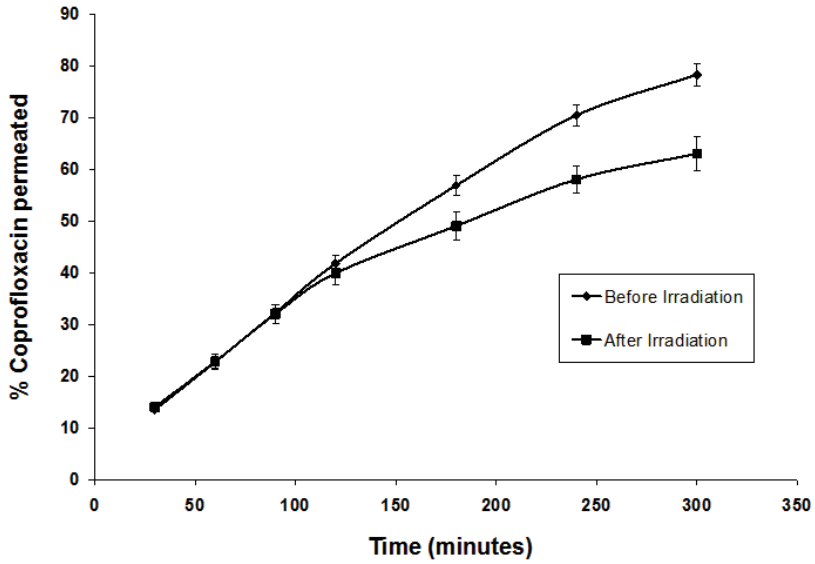

Fig. 6: Release of ciprofloxacin from irradiated (20 kGy) and unirradiated pluronic gel (25% w/v).

In conclusion, the use of gamma irradiation in sterilization of ophthalmic CFX pluronic gels should be under a precise controlled condition as irradiation can affect both physical and chemical properties of CFX pluronic gels especially at high irradiation doses. Pluronic gel of concentration 25% w/v containing 0.3 % CFX could be a sterile product with an acceptable property after gamma irradiation with 20 kGy irradiation dose. Formulation of the irradiated pluronic gel for ocular administration with optimized pH and osmotic pressure for an in vivo study will be further investigated in future.

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