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STABILIZATION OF PREDNISOLONE IN OPHTHALMIC GELS DURING STERILIZATION BY GAMMA RADIATION.

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ABSTRACT
The effect of different types of gel forming polymers, namely Methyl cellulose (MC), Hydroxypropyl methyl cellulose (HPMC), Polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP), on Prednisolone sensitivity to gamma radiation was investigated. At low polymer concentration, irradiation up to 20 KGY resulted in slight protection of the drug. At gel forming concentrations, the drug degradation by radiation was nearly the same as in low polymer so. Moreover, the gel viscosity was considerably decreased by radiation to fluid state in cases of MC and HPMC, while increased in cases of PVA and PVP. The effect of polymer cross-linking on drug stability to radiation was also studied. It was found that 41-49% of initial drug concentration was protected after exposure to 20 KGy of radiation. Also, the maximum polymer concentration which produced the highest cross-linking ratio, while maintaining the gel consistency, was found to be 5%, 4%, 2% and 2% w/w for MC, HPMC, PVA and PVP respectively. Above such concentrations, phase transition to rigid mass was observed. The study also revealed that at high concentration of tween 80 (35%w/w), up to 8%w/w of MC or HPMC and 5%w/w of PVA or PVP could be incorporated in the gels and a significant protective effect was achieved by the four types of polymers. However the highest protective effect was afforded by HPMC.(95%) followed by PVA (93%), MC (90%) and PVP (62%). It can be concluded that the protective effect of the cross-linked polymers was determined by the net result of three factors: the cross-linking ratio; the polymer concentration and the hydrophobicity of the matrix.

KEYWORDS: Prednisolone; gamma radiation; polymeric gels; stability; crosslinking.

INTRODUCTION
Drug delivery in ocular therapy is a difficult task. Poor bioavailability of drugs from conventional ocular dosage forms is mainly due to the pre-corneal loss factors, which include tear dynamics, insufficient residence time in the conjunctival sac and non-productive absorption.[1,2]

In order to improve ophthalmologic bioavailability of drugs and to lengthen the therapeutic action, introduction of semisolid delivery systems[3] and enhanced vehicle viscosity have been proposed.[4]

Polymers are large molecules consisting of repeated small chemical units or monomers. Usually, the repetition is linear, but there are other types wherein chains are branched or interconnected to form three-dimensional networks.[5]

Irradiation of polymeric materials generates some effects depending on the kind of polymer, parameters of irradiation and the state of the material under processing, etc. The two main reactions, which determine the final properties of the polymer, include (a) scission of the main chain, leading to diminishing of the molecular weight of macromolecules and (b) cross-linking, the opposite process ending with macroscopic, three-dimensional material.[6] The yields of scission and crosslinking, or more precisely the mutual ratio of these two parameters, determine the physical properties of the product. Irradiation of water-soluble polymers in aqueous solution is often a method for production of hydrogels[7,8], where the polymeric network exhibits the ability to absorb and retain a significant amount of water within its structure, but not dissolving.[9,10] Hydrophilicity of the polymer used for the formation of hydrogel is of great importance for the final properties of the product.

Cross-linked natural polymers can be used as hydrogel wound dressings, face cleaning cosmetic masks and adsorbents of toxins, while low molecular weight products show antibiotic, antioxidant and plant-growth promoting properties. These successes clearly indicate that radiation processing of natural polymers is an exciting new area where the unique characteristics of these polymeric materials can be exploited for a variety of practical applications.

Cross-linking density, the average molecular mass between the two junction points, affects water absorption characteristic of the hydrogel. In the synthesis of gels by chemical methods, cross-linking density is controlled by the concentration of this cross-linking agent, reaction time, temperature and others. While, for radiation method it is determined by the absorbed dose of radiation. Moreover, cross-linking by the chemical method can be performed only in liquid state, while ionizing radiation, due to its high penetration power, initiates chemical reactions in liquid or in solid state.[11]

Gels are excellent vehicles for several routes of administration. They are useful as liquid formulations in ophthalmic, oral, topical, vaginal, and rectal administration. Gels can be prepared in clear form when all of the particles completely dissolve in the dispersing medium. But this doesn't occur in all gels, and some are therefore turbid. In the gel network, the movement of the dispersing medium is restricted by the dispersed phase, and the viscosity is increased.[12] The cross-linking ratio is one of the most important factors that affect the swelling of hydrogels. Highly cross-linked hydrogels have a tighter structure, and lower swelling compared to the same hydrogels with lower cross-linking ratios.

The chemical structure of the polymer may also affect the swelling ratio of the hydrogels. Hydrogels containing hydrophilic groups swell to a higher degree compared to those containing hydrophobic groups. Hydrophobic groups collapse in the presence of water, thus minimizing their exposure to the water molecule. As a result, the hydrogels will swell much less than hydrogels containing hydrophilic groups.[13] Many authors investigated the effect of radiation dose, molecular weight, polymer concentration, and the influence of the nature of the solvent on cross-linking of polyvinylpyrrolidone, methylcellulose, hydroxy ethylcellulose, hydroxy propylmethyl cellulose and polyvinyl alcohol.[14, 15]

The objective of this research will be focused on the stability of Prednisolone to gamma radiation, during sterilization, in ophthalmic gels prepared from different types of polymers. The study will include; The effect of the types of gel-forming polymers, the effect of polymer concentration and cross-linking ratio, in addition to the protective role of surfactant, at high concentration above CMC.

MATERIALS AND METHODS

Materials

Prednisolone (Pd) and prednisolone acetate were kindly provided from: Julphar, Gulf Pharmaceutical Industries (Ras Al Khaimah, U.A.E). Acetonitrile and methanol Chromasolv®, 99.9% HPLC grade were purchased from Sigma-Aldrich, Chemie GmbH (Steinheim, Germany). Tween 80 was purchased from Merck KGaA (Germany). Methylcellulose (MC), Methocel A4C, Dow Chemical Company (USA), was provided from: Tabuk Pharmaceutical Manufacturing Co. (Tabuk, K.S.A). Hydroxy propylmethyl Cellulose (HPMC), Hypromellose, K-15M, Colorcon (UK) and Polyvinyl alcohol (PVA), M.Wt.= 20 000 were provided from: Riyadh Pharma Medical & Cosmetic Products Co. Ltd. (Riyadh, K.S.A). Polyvinylpyrrolidone (PVP), Luviskol® K-90, was purchased from BASF (Aktiengesellschaft Ludwigshafen, Germany).

HPLC assay of Prednisolone

Pd was assayed by the method described by Gorog S[16] using HPLC chromatographic system, Shimadzu LC-10AD integrated with a class-LC10 system, Kyoto, Japan, equipped with Shimadzu UV-Visible detector and an auto-injector system (SIL-10ADVP Shimadzu auto-injector, Kyoto, Japan.) using reversed phase column Symmetry, C_{18} (4.6 mm× 250 mm), packed with 5 µm particles, maintained at 40 ^{o}C. The mobile phase consisted of a mixture of Acetonitrile and Water in a ratio of (45:55), filtered through 0.45 µm millipore filter and degassed. The flow rate was 1 ml/min and the injection volume was 20 µl. Prednisolone acetate was used as internal standard.

Irradiation of Prednisolone in 1%w/w polymer solutions

1%w/w aqueous solutions of the suggested polymers: MC, HPMC, PVA and PVP were prepared containing 4.028 x 10^{-4} M Pd. Five g samples of each solution were. irradiated with different doses of gamma radiation (1, 2, 3, 5, 10, 15 and 20 KGy) in sealed glass vials. Then the samples were diluted to 20 g with water and the residual drug concentration was determined by HPLC method. The obtained data are illustrated in Table 1.

Stability of Prednisolone to radiation in polymeric gels

Different polymeric gels were prepared containing the minimum amounts of polymers required for gel formation; 4%w/w MC, 2%w/w HPMC, 2%w/w PVP and 2%w/w PVA. All the gels contained the same concentration of the drug (4.028x10^{-4} M). Five g samples were irradiated as before and the residual drug concentrations were determined after proper dilution with water. The obtained results are presented in Table 1.

Table 1: Initial degradation rate (IDR %/KGy) of prednisolone in 1% w/w of polymer solutions and polymeric gels (4%MC, 2% HPMC, 2% PVA and 2% PVP).

Type of Polymer	IDR (%/ KGy) ±SD		
	In aqueous solution	In 1%w/w polymer solutions	In polymeric gel
Control	-16.716 (±0.12)	---	---
MC	---	-8.526 (±0.22)	-7.759 (±0.21)
HPMC	---	-8.350 (±0.02)	-7.845 (±0.43)
PVA	---	-9.26 (±0.14)	-7.055 (±0.24)
PVP	---	-6.970 (±0.11)	-8.076 (±0.72)

Determination of the cross-linking dose of radiation in solid state polymers

Cross-linking was carried out by irradiation of the polymer powders with different doses of gamma radiation, 20, 30 and 50 kGy, then the gels were prepared using the irradiated powders and the same drug concentration was incorporated. Five g samples of the gels were subjected to different doses of radiation as usual. The residual drug concentrations were determined after proper dilution with methanol and centrifugation at 5000 RPM for 5 minutes to separate any undissolved cross-linked polymer. The data are listed in Table 2.

Table 2: Initial degradation rate (IDR %/KGy) and percent of residual concentration of prednisolone in polymeric gels prepared from polymer powders previously exposed to different doses of radiation (A = 20 KGy, B =30 KGy and C = 50 KGy).

Type of polymer	A		B		C	
	IDR ±SD	Res. Drug (% ±SD)	IDR ±SD	Res. Drug (% ±SD)	IDR ±SD	Res. Drug (% ±SD)
MC (4%)	-3.980 (0.59)	47.90 (0.55)	-3.880 (0.54)	49.16 (0.93)	-3.85 (0.88)	48.85 (0.63)
HPMC (2%)	-4.168 (0.78)	43.12 (1.13)	-4.370 (0.84)	42.53 (0.84)	-4.270 (0.79)	40.72 (0.55)
PVA (2%)	-3.798 (0.56)	45.90 (0.85)	-3.754 (0.66)	46.16 (1.15)	-3.342 (0.33)	46.05 (0.88)
PVP (2%)	-3.819 (0.83)	46.98 (1.45)	-3.644 (0.46)	47.16 (1.21)	-3.520 (0.64)	48.05 (0.94)

IDR ±SD of irradiated aqueous drug solution (control) is -16. 716 (±0.12) and Res. Drug % is 0

Determination of the critical cross-linking concentrations of the polymers

Different concentrations of the polymers in aqueous medium were prepared and subjected to a dose of radiation of 20 kGy. The viscosity of each concentration was measured before and after irradiation. The critical cross-linking concentration is the concentration at which the viscosity of the gel increases to the extent of formation of rigid mass. The results are illustrated in Table 3.

Table 3: Viscosity values (determined at 5 rpm) of gels containing different concentrations (%) of polymers, before (A) and after (B) irradiation with 20 KGy for determination of the respective critical cross-linking concentrations.

Polymer Type	Viscosity (cP)											
	1% *		2% *		3% *		4% *		5% *		6% *	
	A	B	A	B	A	B	A	B	A	B	A	B
MC			100	100	225	125	2300	200	10500	17000	13300	Rigid
HPMC			7100	100	10300	130	35500	97000	65000	Rigid		
PVA	100	75	175	12000	200	Rigid						
PVP	175	100	350	11500	1000	Rigid						

* = *Polymer concentration.*

Measurement of gel viscosity

Using Brookfield Viscometer (RV-DV-I Prime with sample chamber SC4-7R and SC4-13R and spindles SC4-15 and SC4-21, Brookfield, USA), the gel sample was placed in the suitable vessel and the proper spindle was selected according to the viscosity of samples. Spindles No. 21 was used for viscous liquids to very light gels, while spindles No.15 was used for highly viscous gels. The RPM was gradually increased from 0.5 up to 50 and the equivalent viscosity was registered.

The effect of tween 80 on critical cross-linking concentrations of polymers

The effect of 35%w/w tween 80 on the critical cross-linking concentration of the polymers was first tested through following up gel viscosity. Up to 8%w/w for MC and HPMC and 5%w/w for PVA and PVP maintained their gel consistency after irradiation in presence of 35%w/w tween 80. Above such polymer concentrations, the irradiated gels changed to rigid masses. The data are presented in Figures 1, 2, 3, 4 and 5.

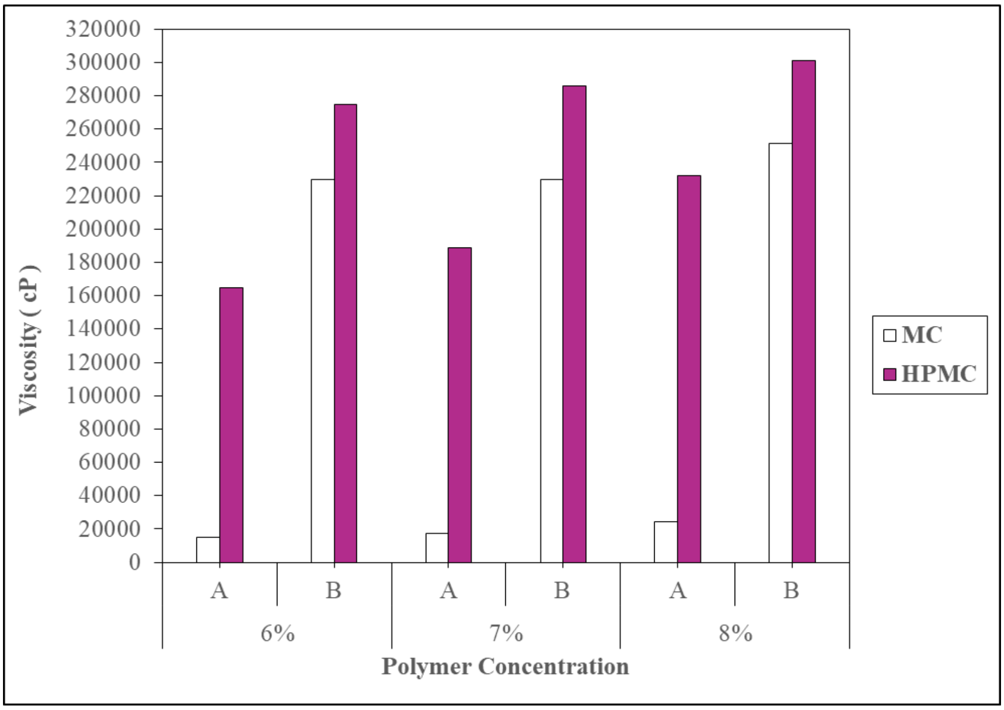

Fig. 1: Viscosity of different concentrations of MC and HPMC gels containing 35%w/w tween 80 (measured at 0.5 rpm) before (A) and after (B) irradiation with 20 KGy.

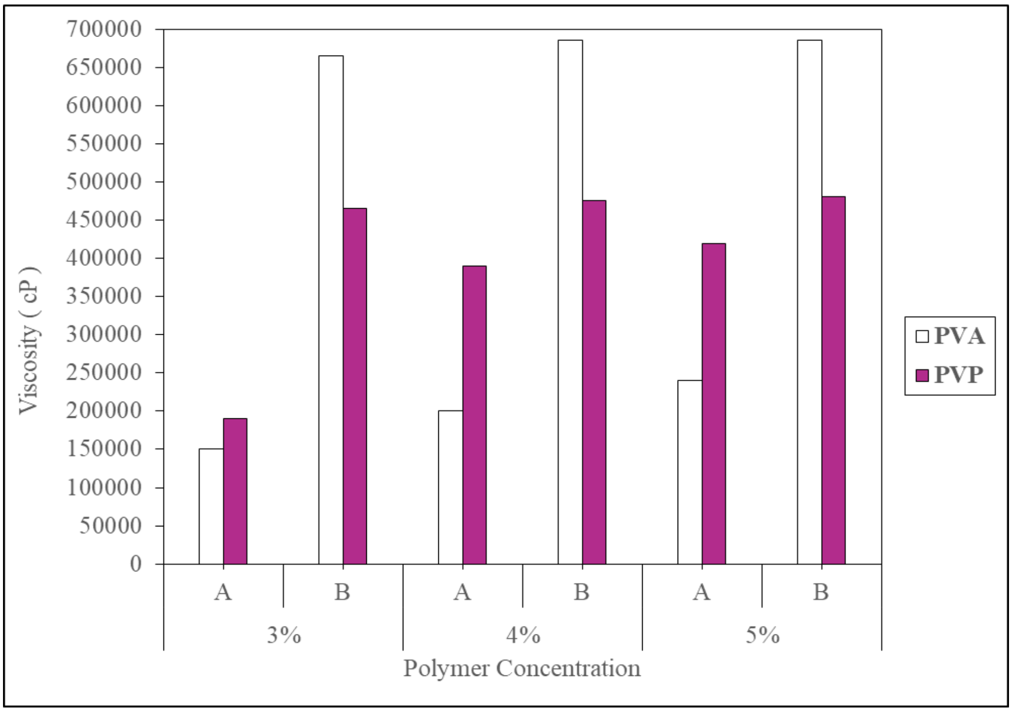

Fig. 2: Viscosity of different concentrations of PVA and PVP gels containing 35%w/w tween 80 (measured at 0.5 rpm) before (A) and after (B) irradiation with 20 KGy.
(*Viscosity values of A are multiplied by 100*).

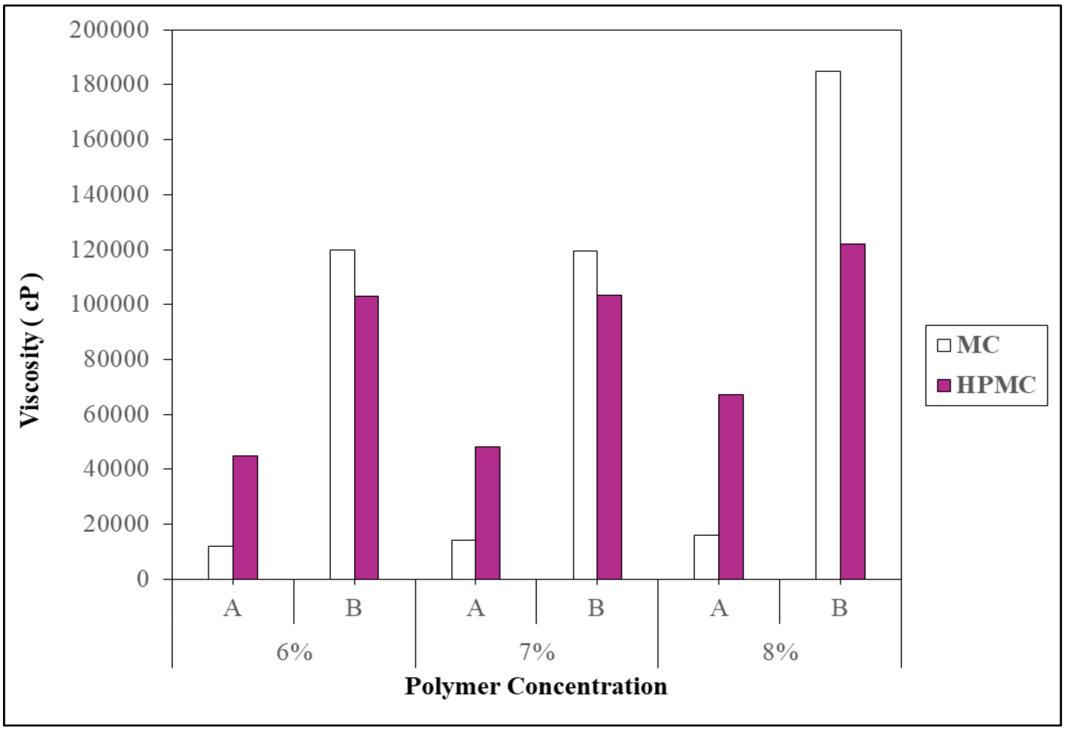

Fig. 3: Viscosity of different concentrations of MC and HPMC gels containing 35% w/w tween 80 (measured at 5 rpm) before (A) and after (B) irradiation with 20 KGy.

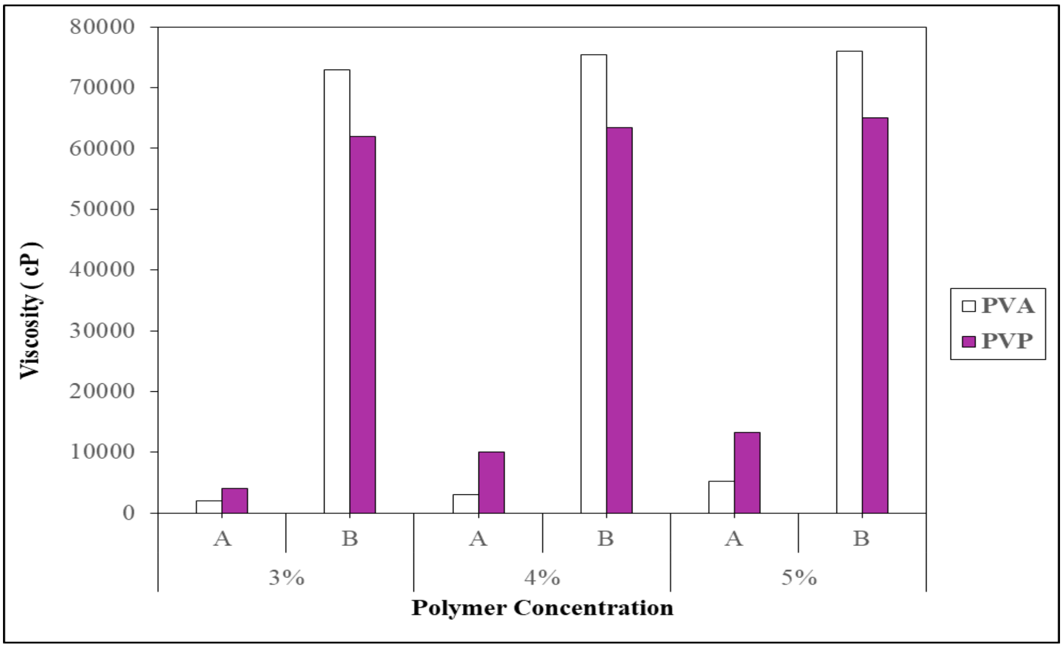

Fig. 4: Viscosity of different concentrations of PVA and PVP gels containing 35% w/w tween 80 (measured at 5 rpm) before (A) and after (B) irradiation with 20 KGy.
(*Viscosity values of A are multiplied by 10*).

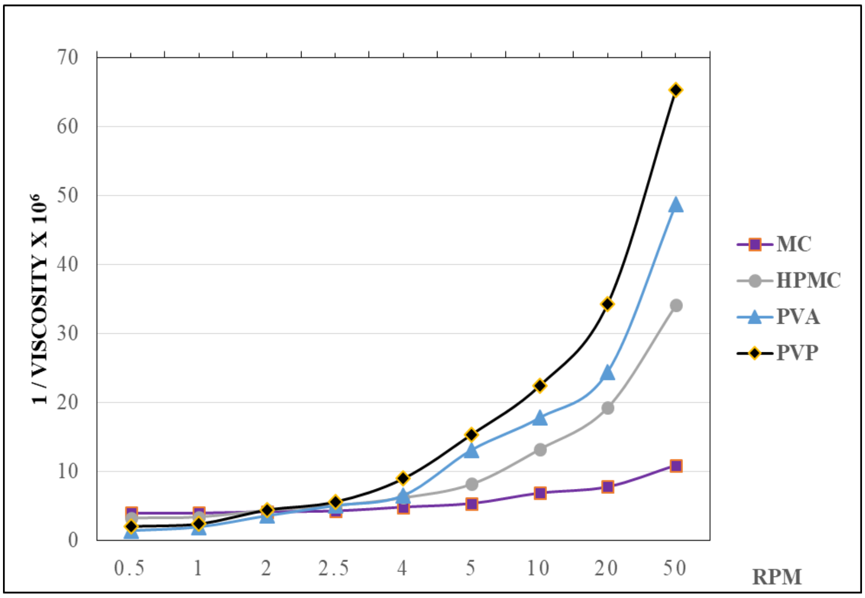

Fig. 5: Flow profile of different polymeric gels (8%w/w MC&HPMC, 5%w/w PVA&PVP) in presence of 35% w/w of tween 80 after irradiation with 20 KGy.

The effect of tween 80 on drug stability to radiation
Polymeric gels (8%w/w for MC, HPMC and 5%w/w for PVA, PVP) were prepared containing 35%w/w tween 80 and 4.028×10^{-4} M Pd. One g gel samples were irradiated with different doses of radiation and the residual drug concentrations were determined by HPLC after proper dilution with methanol and centrifugation at 5000 RPM to separate any un-dissolved cross-linked polymer. The obtained data are presented in Table 4 and Figure 6.

Table 4: Degradation rate (%/KGy) and residual concentrations (%) of prednisolone in polymeric gels containing 35%w/w tween 80.

Type of polymer	Degradation rate (%/KGy) ±SD	Residual drug concentration(%) ±SD
Tween 80 (alone)	-1.043 (±0.35)	79.85 (±1.44)
MC (8%)	-0.340 (±0.73)	90.67 (±1.55)
HPMC (8%)	-0.203 (±0.57)	95.86 (±1.57)
PVA (5%)	-0.302 (±0.84)	92.97 (±0.58)
PVP (5%)	-1.895 (±0.83)	63.77 (±1.59)

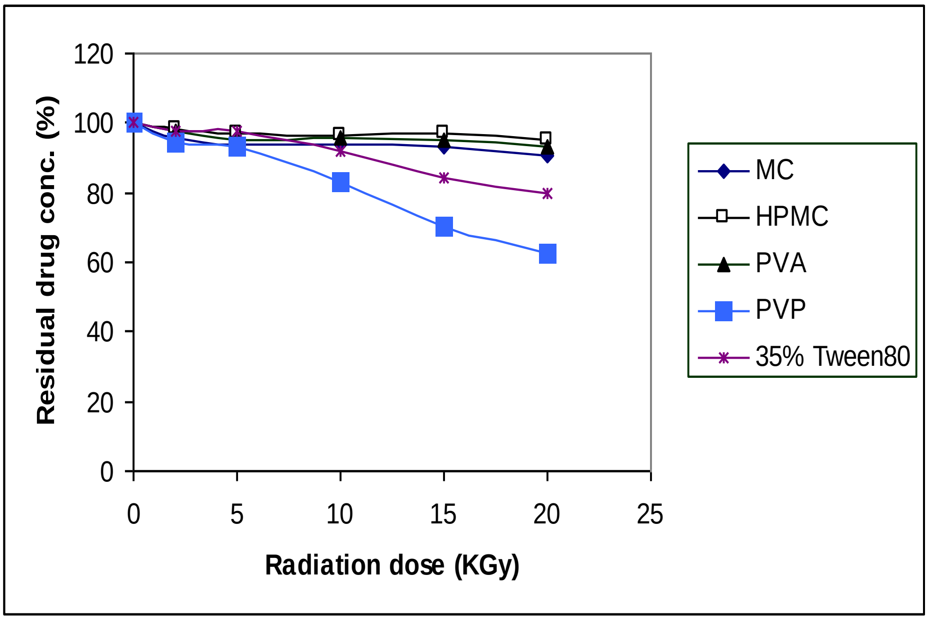

Fig. 6: Stability of prednisolone to gamma radiation in polymeric gels (8% MC, 8% HPMC, 5% PVA and 5% PVP). containing 35%w/w tween 80.

RESULTS AND DISCUSSION

It is obvious from Table 1 that all the polymers had a slight stabilizing effect on the drug. At such low concentration of the polymers, irradiation resulted in scission of their molecular structure, leading to diminishing of the molecular weights and production of fractionated species.[11] In other words, the polymer molecules compete with the drug for interaction with the radiolytic products of water. The net result is the indirect slight protection of the drug. The trend of higher protective effect of PVP compared to other polymers may be attributed to its higher reactivity to the radiolytic products of water and acts as free radical scavenger.

It was expected that the gel network structure at higher polymer concentrations would protect the drug to certain extent from radiation. However, the drug degradation by radiation was nearly the same as in 1% polymer solutions. Moreover, the gel viscosity was considerably decreased by radiation to nearly fluid state in cases of MC and HPMC, while increased in cases of PVA and PVP. It was reported by **Kiran et al**[17] that the dependence of reduced or increased viscosity of PVA and PVP polymers on their concentrations and absorbed radiation dose showed three distinct regions which were associated with intramolecular cross-linking and chain scission (decrease in viscosity), intermolecular cross-linking (increase in viscosity) and intramolecular cross-linking (decrease in viscosity) via ring formation. This would explain the observed decreased viscosity of gels prepared from MC and HPMC, while increased in PVA and PVP, after irradiation.[18] Nevertheless, the intermolecular cross-linking, which is responsible for the increasing viscosity in cases of PVA and PVP, was not sufficient to protect the drug from radiation and at the same time, drug degradation was taking place in parallel with the cross-linking process. Consequently, the protective effect of all types of polymers was very weak, which resulted in degradation of about 92- 95% of the drug after exposure to 20 kGy of radiation as shown in Table 1.

Pekel et al[19] reported that cellulose and their derivatives irradiated with ionizing radiation in solid state or in aqueous systems resulted in a degree of cross-linking depending on polymer concentration as well as radiation dose. Therefore, exposing the polymer powder to different doses of radiation would be expected to result in increasing the extent of cross-linking (cross-linking ratio). The extent of cross-linking of different types for polymers could be followed up by their viscosities as well as the stabilizing effect to the drug to radiation when prepared in gel form. Therefore, gels were prepared from the previously irradiated polymer powders, with different doses of radiation, in concentrations 4%, 2%, 2% and 2% of MC, HPMC, PVA and PVP respectively, and the drug was incorporated at constant concentration. The results presented in Table 2 show an improved stabilizing effect, for the drug to radiation, ranging from 41% - 49% of the initial drug concentration. This means that cross-linking of the polymers showed a significant stabilizing effect for the drug to radiation. This could be attributed to the inclusion of the drug inside the cross-linked polymeric matrix, formed through irradiation of the polymer powders. This would result in protection of the drug from the attacking species. The results illustrated in Table 2 reveal no significant difference ($P > 0.05$) between the three doses of radiation for the same polymer, indicating that 20 kGy is considered to be a sufficient dose for maximum cross-linking of the polymer powder. It is also evident that the four types of polymers showed no significant differences ($P > 0.05$) in their stabilizing effects to the drug.

Polymer concentration is a critical factor in determining the extent of cross-linking by radiation.[20] Therefore, different polymer concentrations were irradiated with 20 kGy (a sufficient radiation dose for maximum cross-linking of polymer powders). As the viscosity is considerably affected by cross-linking (either decreases by intramolecular cross-linking or increases by intermolecular cross-linking), the viscosity of the gels were measured before and after irradiation. Table 3 illustrates that the maximum polymer concentration producing the highest cross-linking ratio, while maintaining the gel consistency, was found to be 5%, 4%, 2% and 2% for MC, HPMC, PVA and PVP respectively. Above such concentrations, the gel was changed to a rigid mass by radiation. The rigid mass indicates the formation of highly cross-linked structure characterized by low water solubility.

To prepare gels with higher polymer concentrations than the critical cross-linking concentration, while maintaining their gel consistency, a high concentration of tween 80 (35% w/w) was added during the preparation of the gels. **Chan et al**[21] reported the safety of using up to 40% tween 80 in eye drops. Tween 80 would be expected to have two functions: The first is the interference with the cross-linking process by formation of hydrogen bonding at the sites of cross-linking, by the OH groups of the surfactant, leading to incorporation of a higher amount of the polymer before reaching the maximum cross-linking. This suggestion is in agreement with **Rosiak et al**[22] who reported that glycerol interferes with the cross-linking of PVP through formation of hydrogen bonding at the sites of cross-linking in the polymer. Secondly, the presence of tween 80 in a high concentration would be expected to afford further protection to the drug through micelle formation.

The results illustrated in Figures 1 and 2 reveal that up to 8%w/w of MC or HPMC and 5%w/w of PVA or PVP could be incorporated in the gels with 35% w/w of tween 80. It can be also noted that a higher viscosity was reached by PVA and PVP compared to MC or HPMC at low shearing rate (0.5 RPM) which would indicate that a higher cross-linking ratio occurred in these polymers. This could be explained on the basis that PVA has one

OH group for every two carbons of the vinyl unit of the polymer leading to a very high number of cross-linking sites. Therefore a high cross-linking ratio can be obtained at low concentration of the polymer. The same explanation could be applied to PVP which has a nitrogen atom (ready to be quaternary by hydrogen bonding) in addition to the oxygen atom of the carbonyl group in each unit of the polymer. In cases of MC and HPMC, the ratio of cross-linking sites to molecular weight is lower than in PVA or PVP due to the higher number of carbon atoms in each cross-linking unit.[11] On the other hand, at a higher shear rate (5 RPM) the viscosities of PVA and PVP were lower than that of MC and HPMC (Fig. 3 and 4) which could be attributed to the higher mobility of PVA and PVP molecules due to their smaller size.

Figure 5 shows the flow profile of the four polymers in presence of tween 80 with shear thinning pattern, where the viscosity decreases by increasing the RPM. This would permits the gradual drug release as the gel is applied in the eye. The lower response of MC and HPMC to increasing RPM compared to PVA and PVP may be attributed to the larger and tight network structure of the cross-liked polymer.

It is clear from Table 4 and Figure 6 that a significant protective effect was achieved by the four types of polymers. However the highest protective effect was afforded by HPMC. This could be attributed to the high cross-linking ratio of HPMC compared to that of MC (due to the extra OH group). On the other hand, the glycoside ring and the larger number of carbon atoms in HPMC, makes the cross-linked units of HPMC more hydrophobic than PVA and PVP. This would also explain the lower protective effect of PVP (only 62%) which could be due to the hydrophilic nature of the cross-linked units compared to those of MC and HPMC. It can be concluded that the protective effect of the cross-linked polymers is determined by the net result of three factors: The first is the cross-linking ratio; the second is concentration of the polymer in the gel and third is the hydrophobicity of the matrix.[1,2]

CONCLUSION

Irradiation of low concentration of polymers (1%w/w) resulted in scission of polymer molecules and the fractionated products had a very slight protective effect on drug through scavenging mechanism.

At gel-forming concentrations, irradiation significantly decreased the viscosity of the gel due to intramolecular crosslinking and scission mechanisms. The protective effect of the irradiated gels was nearly the same as that of the dilute solutions.

Crosslinking of polymers depends on the polymer concentration (critical crosslinking concentration) and the dose of radiation. The critical crosslinking concentration (cross-linking ratio) can be followed up through the increase of viscosity.

Gels prepared from previously cross-linked polymer powders, by exposure to 20 kGys of radiation, had a significant protective effect on drug protection against radiation accompanied with considerable increase in gel viscosity. This could be attributed to the inclusion of drug inside the cross-linked structure of the gel.

Addition of a high concentration of tween 80 (35%) interferes with crosslinking process through hydrogen bonding with the polymer molecules at the sites of crosslinking, leading to increasing the critical crosslinking concentration. This in turn allows the inclusion of a higher concentration of polymers before reaching the maximum crosslinking ratio and phase transition (formation of rigid mass). Consequently, a higher protection to drug was afforded.

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