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Adaptation of Hard Gelatin Capsules for Aqueous Solution Delivery Using Gamma Radiation

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ABSTRACT

Objective: Directly incorporating aqueous solutions into hard gelatin capsules (HGCs) without dispersing them in an oily medium is considered a challenge for most researchers and manufacturers. The aim of the study is to evaluate the effect of gamma radiation (ɣ-radiation) on the adaptation of HGCs for aqueous solution delivery. **Methods:** Empty HGC shells were exposed to four of ɣ-radiation doses (1, 3, 5, 10 kGy). Then, the physicochemical properties of irradiated capsules were evaluation and compared with those of non-irradiated capsules. Fourier-transform infrared spectroscopy (FT-IR), capsule hardness, and water incorporation tests were performed. *In-vitro* disintegration/dissolution behavior determined as (rupture time) in different dissolution media was evaluated. **Results:** The results showed direct proportionality between the ɣ-radiation dose and HGC crosslinking degree up to 3 kGy, while at doses >3 kGy, degradation rather than crosslinking occurred. The results were clearly demonstrated by FTIR as peptide linkages between gelatin molecules. All the ɣ-irradiated HGCs submitted to hardness test were completely deformed without rupture with increasing capsule deformation work (J) for γ-radiation doses up to 3 kGy; the deformation work declined at doses >3 kGy. The water incorporation study revealed that capsules exposed to 3 kGy could hold up to 100 ml of methylene blue solution without deformation or leakage for 45 minutes compared with non-irradiated HGCs, which showed a significantly lower tolerance of only 2 minutes (p<0.001). The crosslinking of HGCs had a minor significant effect on *in-vitro* rupture time, especially at gastric pH. **Conclusion:** The irradiation technique may be used not only for sterilizing HGCs but also for adapting HGCs for aqueous solutions delivery, as it showed a significant positive effect, which was optimal at a dose of 3 kGy. However, these results are not sufficient for scaled-up manufacturing; thus, further investigations are strongly recommended.

Keywords: *Cross linking; Gamma radiation; Hard gelatin capsules; Liquid-filled capsules*

INTRODUCTION

Recently, developments in the fields of formulation science and technology have offered new methods for filling hard gelatin capsules (HGCs) with oil-soluble liquids and semi-solid formulations for oral drug delivery[1]. The interest in delivering aqueous solutions through HGCs has been leveraged to incorporate highly potent agents, hormones, cytotoxic drugs and radiopharmaceuticals with high uniformity,

minimal work hazards and minimal loss of chemicals[2,3]. However, there are many barriers to achieve such formulations. The impact of liquid fillers on the mechanical properties of HGCs must be considered when developing such formulations. It is known that such solvents may cause the gelatin shell to become either brittle or soft. Hydrophilic substances may be formulated into capsule filler, but water or low molecular weight alcohols should be kept at or below 10% w/w. Higher levels will initiate erosion and softening of the shell because of diffusion and evaporation from the fill material and/or the shell[4]. Gelatin crosslinked via a wide variety of chemical and physical crosslinking techniques has been extensively studied for long-term biomedical applications because crosslinking this biopolymer elevates its thermal and mechanical stability and improves its water resistance[5,6]. Among the different physical crosslinking methods, irradiation has become well known as a very convenient tool for improving the mechanical properties, chemical resistance, thermal stability, melt flow and other important properties of polymeric materials[7]. Radiation processing has also become a well-accepted technology worldwide, with diverse uses ranging from the irradiation and curing of food products[8], to the sterilization and surface modification of medical devices[9]. Exposing a polymer to radiation, especially ionizing radiation (gamma; ɣ), can lead to ionization and excitation, chain scission, or crosslinking and changes in bulk and surface properties[10,11]. Gamma rays are emitted from a nuclear source, such as ^{60}Co or ^{137}Ce. One major benefit of radiation-induced crosslinking is that the irradiated candidate can be ingested safely with no residual chemical reagents, such as toxic crosslinkers (formaldehyde, glutaraldehyde)[12]. Irradiated gelatin is promising for use in many applications in various fields, according to many studies[13]. A study performed by Zaman et al. (2013) determined that gamma radiation (ɣ-radiation)-treated gelatin bioadhesive films showed higher tensile strength and elongation at break than untreated films[14].

Aim of the work: This work focused on studying the effect of ɣ-radiation on increasing HGC tolerance to being filled with aqueous solutions. A wide range of radiation doses were used (1–10 kGy).

MATERIALS AND METHODS

Empty HGC shells (size 0) were generously provided as a gift by Capsugel, Colmar/Strasbourg, France. Methylene blue (MB) was purchased from EL-Gomhouria Company, Cairo, Egypt.

ɣ-Irradiation of HGC shells

HGCs were inserted into glass bottles, sealed and subjected to ɣ-rays from a ^{60}Co source (National Centre for Radiation Research and Technology [NCRRT], Egypt) at doses of 1, 3, 5 and 10 kGy, producing samples F2-F5, respectively. The radiation process was performed in the presence of nitrogen and at ambient temperature.

Characterization of ɣ-irradiated and non-irradiated HGC shells

Fourier-transform infrared spectroscopy (FTIR)

FTIR (Shimadzu 8400S, Lab Wrench, Japan) was employed within the spectral region of 400–4,000 cm^{-1} to interpret the conformational and structural changes of protein molecules in the HGCs during irradiation process. The method was adopted as previously described[15].

Capsule hardness

The hardness of non-irradiated (F1) and ɣ-irradiated HGCs was obtained via the method reported by Cilurzo et al.(2005)[11] using a tensile testing apparatus (Zwick/Roell Tensile Testing Proline, Germany). Each capsule was compressed at a constant rate of 10 mm/min until the shell was broken or completely compressed. The hardness of HGCs could be interpreted as capsule deforming work (J)[16]. The work required to deform the capsule was determined. The results are expressed as the mean ± SD of 6 samples.

Effect of ɣ-radiation dose on capability of ɣ-irradiated HGCs to be filled with aqueous solution

ɣ-Irradiated HGCs were filled with five different volumes of an aqueous solution (10, 25, 50, 100, 250, 500 µl) to evaluate the tolerance of the capsules for water incorporation and determine the maximum fill volume achievable without causing any leakage or capsule deformation. As the judgement was visually determined, a coloured aqueous solution (2% w/v MB) was selected to accurately indicate any leakage, which signified as the water uptake capacity of the HGCs.

Effects of dissolution medium and ɣ-radiation dose on HGC *in-vitro* rupture time

The dissolution method used for filling HGCs with a hydrophilic fill material was previously developed. Disintegration could be used as a performance indicator if the drug is already dissolved in the hydrophilic matrix because shell rupture is the limiting step. Rupture is a more relevant process than complete disintegration, which usually measures dissolution of the shell. For water-based formulations containing concentrated solutions, release of the capsule content by shell rupture may be the only relevant step for determining the bioavailability and the *in vivo-in vitro* correlation (IVIVC)[4]. For this reason, several monographs in the

United States Pharmacopeia (USP) contain rupture tests as the only performance standard for these dosage forms[17]. However, dissolution testing should be required if the drug is dispersed as a suspension or emulsion in the hydrophilic matrix. The rupture time of the γ-irradiated and non-irradiated HGCs was determined based on the MB released, as measured using a USP Apparatus 2 paddle dissolution tester. However, baskets (USP Apparatus 1) may not be suitable in certain instances because as a HGC breaks down, the gelatin from the shell may clog the basket's mesh[4].

In this study, 100 µl of 2%w/v MB aqueous solution was filled into capsules and placed in 100 ml of dissolution medium kept at 37ºC under stirring at 100 rpm. Deionized water, 0.1N HCl buffer and phosphate buffer (pH 7.4) were used as dissolution media. At predetermined time intervals, a one-millilitre sample of dissolution medium was withdrawn and replaced with fresh media. The samples were analysed for released MB spectrophotometrically at λ_{664nm} using UV/visible spectroscopy (JASCO spectrophotometer, Japan). The results are expressed as the HGC rupture time for MB release of three replicates.

Statistical analysis

All data are expressed as the mean ± SD and were analysed using the Statistical Package for Social Sciences (IBM SPSS, v 20.0 software, Inc., Chicago IL, USA); one-way ANOVA was applied followed by a post hoc (Dunnett) test for multiple comparisons with F1. Values of $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Characterization of γ-irradiated and non-irradiated HGC shells

FTIR analysis

The FTIR spectra of non-irradiated (F1) and γ-irradiated (F2-F5) HGCs are shown in **Figure 1** and **Table 1**. The FTIR spectrum of F1 shows characteristic bands of gelatin at 3342.13, 2923.71, 1641.72 and 1549.64 cm^{-1}, corresponding to the amide A peak (N–H stretching coupled with the hydrogen bond of a carbonyl group in a peptide chain), the amide B peak (asymmetric stretching vibration of alkenyl C–H and NH_3^+), the amide I peak (C=O stretching vibration of peptide linkages) and the amide II peak (N–H and C–N stretching vibration), respectively.

For both F2 and F3, HGCs subjected to γ-radiation at 1 and 3 kGy, respectively, **Figure 1** and **Table 1** illustrate a shift of the characteristic amide A peak to a higher wave number. However, for F4 and F5, HGCs subjected to γ-radiation at 5 and 10 kGy, respectively, a shift of the characteristic amide A peak to a lower wave number was observed. The shift of the

amide A peak absorption band to higher wave numbers at radiation doses of 1 and 3 kGy (**Figure 1**, **Table 1**) may be attributed to crosslinking and the formation of hydrogen bonds between the amino acid residues of the chains, wherein crosslinking dominates over denaturation[5]. On the other hand, the shift of the amide A peak absorption band to lower wave numbers at higher radiation doses (5 and 10 kGy) could be due to protein molecules degradation dominating over crosslinking at elevated radiation doses[18].

The characteristic amide B, amide I and amide II peaks remained unaffected by the γ-radiation at all used doses. The noted non-significant changes in the amide B, I and II peak values are in agreement with the findings of previous studies, showing that radiolysis in the absence of oxygen (nitrogen atmosphere) does not lead to chain scission or other important means of degradation[19,20].

Capsule hardness

All capsules submitted to hardness testing were completely deformed without shell rupture. The capsule deformation work determined for the non-irradiated HGCs was less than that measured for the γ-irradiated HGCs, as shown in **Figure 2**. Compared with the capsule deformation work of F1 (0.12 J), the capsule deformation work of F2 (0.16 J) and F3 (0.20 J) was significantly increased ($p < 0.01$ and $p < 0.001$, respectively), which was interpreted as an increase in the hardness of HGCs exposed to low radiation doses. However, the capsule deformation work of F4 and F5 (0.13 J and 0.11 J, respectively) was not significantly different from that of F1 ($p > 0.05$).

The results support the FTIR findings, which can be attributed to the predictable contribution of γ-irradiated amino acid residues, wherein physical crosslinking dominates over protein denaturation at radiation doses <5kGy. However, rupture of peptide linkages (degradation) dominates over crosslinking at radiation doses ≥5kGy.
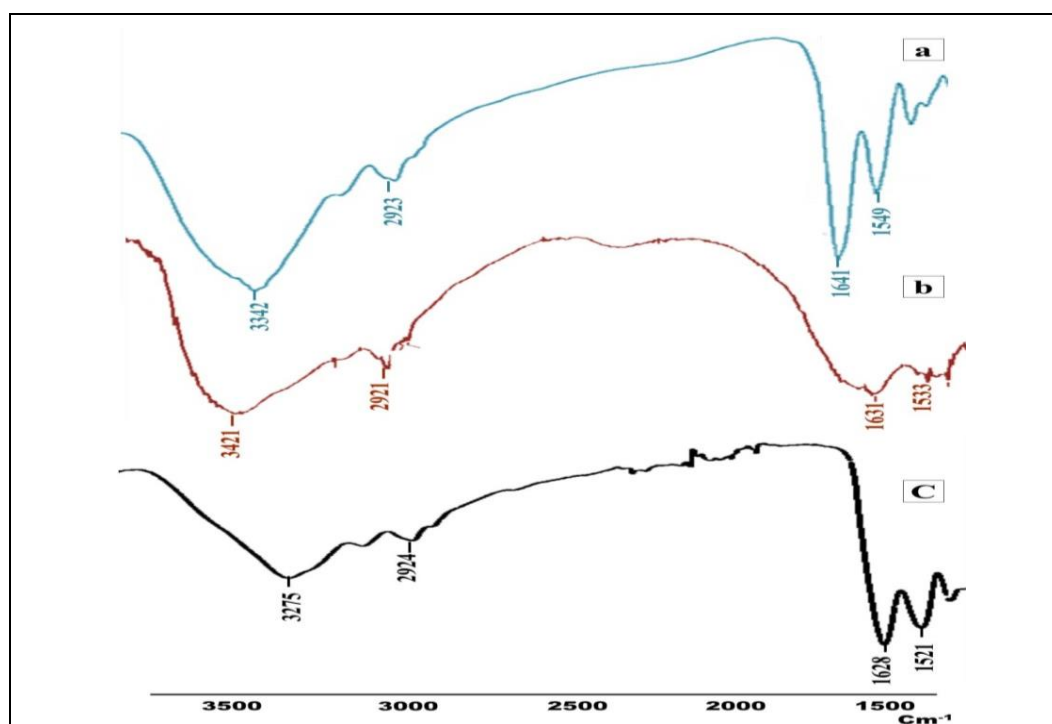
Effect of γ-radiation dose on capability of γ-irradiated HGCs to be filled with aqueous solution

The capability of HGCs to be filled with aqueous solution was determined by the onset of MB leakage (**Table 2**). The non-irradiated capsules showed leakage after only 3.5 min when filled with the smallest volume of MB solution (10 µl). The time to leakage onset decreased with increasing volumes of MB solution, such that abrupt leakage (0 min) occurred when the capsules were filled with 500 µl of MB. Compared with the time to leakage onset for 10 µl of MB, significantly different times were observed for 25 µl ($p < 0.05$) and ≥50 µl ($p < 0.001$) of MB.

On the other hand, there was also a significant difference between the non-irradiated and γ-irradiated

Table 1. Effect of ɣ-radiation dose on the peak wave number (cm⁻¹) of HGC functional groups compared with non-irradiated HGCs.

ɣ-Radiation dose (kGy)	Amide A (cm^{-1})	Amide B (cm^{-1})	Amide I (cm^{-1})	Amide II (cm^{-1})
zero	3342.13	2923.71	1641.72	1549.64
1	3401.68	2924.52	1635.52	1529.13
3	3421.72	2921.09	1631.78	1533.33
5	3282.15	2927.26	1635.65	1528.41
10	3275.62	2924.11	1628.25	1521.03

**Figure 1. FTIR spectra of (a) non-irradiated HGCs (F1) and HGCs subjected to ɣ-radiation at (b) 3 kGy (F3) and (c) 10 kGy (F5).**

HGCs in the capacity to hold MB aqueous solution. Compared with that of the non-irradiated HGCs, the tolerance of the γ-irradiated HGCs to be filled with aqueous solution gradually increased with increasing radiation doses, reaching a maximum at 3 kGy ($p < 0.001$). However, at radiation doses >3 kGy, the capability of the γ-irradiated HGCs to be filled with aqueous solution decreased to a level not significantly different ($p > 0.05$) from that of the non-irradiated HGCs.

The water uptake capacity of both non-irradiated and γ-irradiated HGCs (appeared as HGC shells deformation and swelling) could explain the results obtained for the onset of MB solution leakage, which agree with previous findings[18], showing lower water uptake for γ-irradiated HGCs than for non-irradiated HGCs up to 2.5 kGy. The authors suggested that radiation doses up to 2.5 kGy induced crosslinking along with interconnection among the gelatin pores due to the formation of a three-dimensional network as a function polypeptide chain crosslinking. These effects could reduce the void space within the gelatin structure, inhibiting water from penetrating the HGC shells.

On the other hand, the obtained results suggest a decrease in HGC capacity to be filled with aqueous solution without leakage with the use of radiation doses

Table 2. Effect of γ-radiation dose on capability of γ-irradiated HGCs to be filled with aqueous solution, expressed as the time to MB leakage onset.

γ-Radiation dose (kGy)	Time to leakage onset (min) after filling HGCs with different volumes of MB solution					
	10 µl	**25 µl**	**50 µl**	**100 µl**	**250 µl**	**500 µl**
zero	3.5±1	3±0.5	2.6±0.6	2±0.6	0	0
1	37±4	31±2.5	28±2	25±2.5	3.5±0.5	0.5±0.3
3	55±7	49±4	48±2.5	45±4	5±1	2.5±0.3
5	3.6±0.6	2.6±0.5	2.5±0.5	2.25±0.5	0	0
10	2±0.25	1.6±0.3	1.5±0.5	1±0.5	0	0
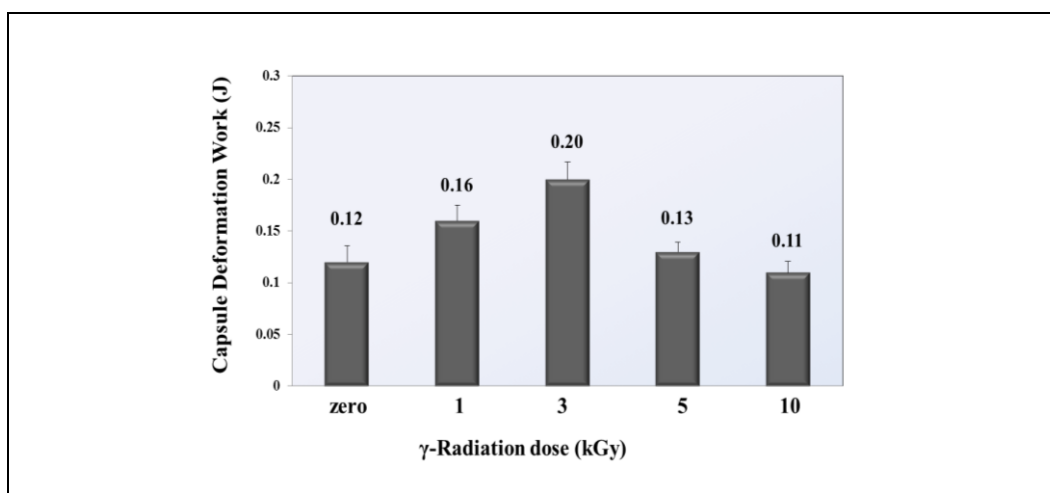
Figure 2. Effect of γ-radiation dose on HGC shell deformation

>3 kGy (**Table 2**). The amide bonding of polypeptide chains within the gelatin molecules determines HGC shell pore size[18]. Thus, these results could be confirmed by the FTIR data. Radiation doses >3 kGy could affect protein molecules by degrading amide chains, leading to increases in pore size and a subsequent increase in water uptake. This mechanism may explain the decrease in the time to the onset of MB aqueous solution leakage from HGCs irradiated with γ-radiation doses of 5 and 10 kGy.

Effects of dissolution medium and γ-radiation dose on HGC in-vitro rupture time

Figure 3 shows the dissolution behaviour, tested as rupture time, of the non-irradiated HGCs (F1) and γ-irradiated HGCs (F2-F5) in deionized water at 37ºC. A lag time was observed, and rupture occurred in the following order: F3>F2>F1>F4>F5. Compared with

the rupture time of F1, high significant differences were observed for the rupture times of F2 and F3 ($p < 0.001$), while non-significant differences were observed for those of F4 and F5 ($p > 0.05$).

The observed delay in the in-vitro rupture time (lag time) of the HGC shells when deionized water was used as the dissolution medium (**Figure 3**) during the dissolution testing could be explained by gelatin crosslinking[16]. This result agrees with that previously reported by Zhang (2010), who showed that some in-vitro dissolution curves of gelatin presented an initial delay of a few minutes due to the aqueous dissolution medium[5]. The crosslinking of the HGCs, due to the action of water, resulted in the formation of rubbery water-insoluble membranes known as pellicles, i.e., thin clear membranes of crosslinked protein surrounding the capsule, which may act as a barrier preventing the filler from being released. This crosslinking involves strong
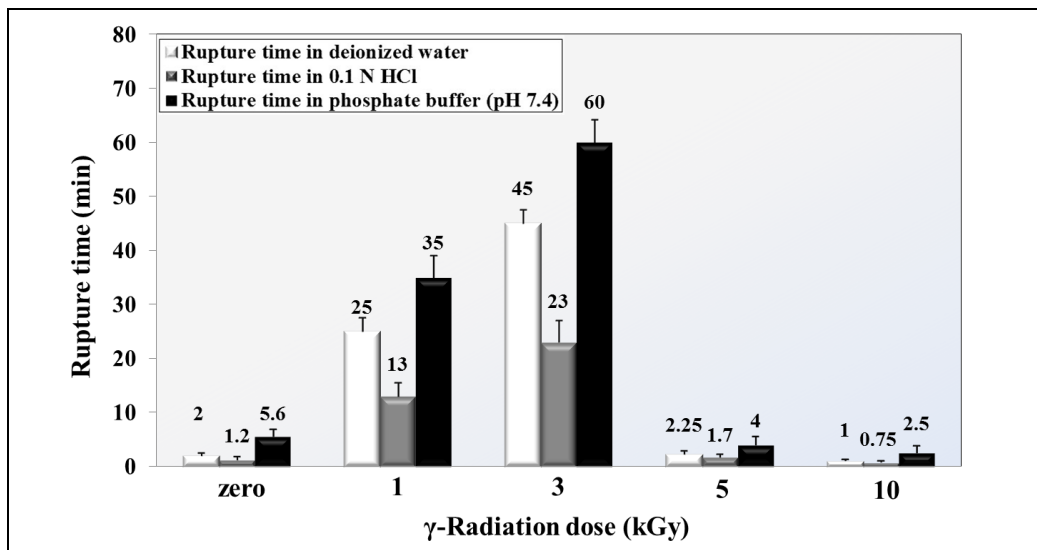
Figure 3. Effect of dissolution medium and γ-radiation dose on HGC *in-vitro* rupture time.

chemical linkages between gelatin chains, via covalent bonding of the amine group of a lysine side chain of one gelatin molecule to a similar amine group on another molecule[21]. Humidity may play a role as a catalyst in the formation of imines, which is the origin of such covalent bonds. Several studies on HGCs have shown decreases in the dissolution degree (in deionized water) after storage in high relative humidity and temperature conditions. The covalent bonding produced by this type of crosslinking is irreversible, and dissolution of the shell must involve breaking the peptide bonds enzymatically or by changing the pH or temperature. Water did not dissolve the HGC shells well at temperatures below $30°C$[11, 22], thus, HGC dissolution was estimated at $37°C$.

The dissolution behaviour of the non-irradiated HGCs (F1) and γ-irradiated HGCs (F2-F5) in dissolution media at different pH values (1.2 and 7.4) is illustrated in **Figure 3**. In medium with an acidic pH, the dissolution time was significantly shorter than that in deionized water, indicating a faster dissolution rate (p<0.001; **Figure 3**). Similar effects of dissolution media on the *in-vitro* rupture time of HGCs have previously been documented[23]. However, longer HGC rupture times were observed n phosphate buffer (pH 7.4) than in deionized water (p<0.001). These results are in agreement with those previously presented by Chiwele et al. (2000), which showed that presence of potassium and/or calcium ions in the dissolution medium significantly delayed the rupture of HGCs containing acetaminophen, while the effect of pH on HGC dissolution was minimal[22]. The behaviour of the HGCs tested in the different dissolution media could be arranged in the same order as in deionized water: F3>F2>F1>F4>F5. Several studies have shown that gelatin protein crosslinking has a detrimental effect on HGC solubility, which may affect the drug's bioavailability[24-25].

As the type-B gelatin used in the study has an isoelectric point (IP) of ≈ 5.5. Previous studies have shown that the minimum solubility of gelatin is at its IP, which designates the pH value of a pure protein in salt-free water. The dependence of HGC shell dissolution on pH has previously been discussed[22]. The solubility of proteins depends to a large extent on pH and the concentration of salts present in solution[26]. As 85–92% of gelatin is composed of proteins, it contains both cationic and anionic groups. The electrostatic interactions in this polyelectrolyte gel are influenced by pH and salt concentrations[27, 28]. Thus, gelatin gels shrink when the pH is close to the IP (5.5) and swell when the pH varies[29]. This behaviour could explain the faster *in-vitro* rupture times (disintegration/dissolution) of HGCs in the acidic dissolution medium.

The greater relative ionic strength of phosphate buffer (as a dissolution medium) reportedly prolongs the swelling behaviour and disintegration time (*in-vitro* rupture time) of HGCs considerably, which could be related to the degree of ionization of the solution; this phenomenon has been attributed to the formation of ion pairs between network charges and counterions[30]. The authors studied the swelling behaviour of gelatin using solutions with different NaCl concentrations. Miyawaki et al. (2003) investigated the effect of water potential on the sol-gel transition and intermolecular interactions of pig skin-derived gelatin[31]. They suggested that during gelation, triple helix formation occurs, involving electrostatic and hydrophobic interactions, as well as hydrogen bonding. The addition of salt can modify these electrostatic interactions and affect the stability of

the gelatin network. Thus, the swelling of a gelatin gel decreases with increasing NaCl content. The authors showed that all hydrogels collapsed as the NaCl concentration increased.

The slower *in-vitro* rupture time of F2 and F3 γ-irradiated HGCs thanF1 non-irradiated HGCs in deionized water (**Figure 3**) is attributable to the polypeptide chain crosslinking induced by the γ-radiation, which inhibited the water from entering the HGCs[18]. However, at radiation doses >3 kGy, faster *in-vitro* rupture times (disintegration/dissolution) were observed as a result of the degradation effect of γ-radiation doses >3 kGy. These results were confirmed by the findings of the water uptake study. Taken together, the results confirm previously reported data showing that the dissolution of HGCs and consequently the bioavailability of the contents are affected by different types of crosslinking[22, 25].

CONCLUSION

This study evaluated the possibility of using radiation to adapt HGCs for the oral delivery of aqueous solutions. The γ-irradiated HGCs (3 kGy) exhibited higher tolerance to aqueous solutions due to physical crosslinking. These γ-irradiated HGCs could incorporate up to 100 µl of water for 45 minutes without deforming or leaking capsule content. *In-vitro* disintegration and dissolution (indicated by rupture time) were not hindered by the crosslinking process. However, these findings are not sufficient for scaled-up manufacturing. Despite the short time tolerated by the HGCs after being filled with aqueous solution (45 minutes), this approach could be applied in hospitals to fill HGCs with radiopharmaceutical preparations, such as aqueous [131]iodine (^{131}I). As capsule deformation occurs with very low volumes of liquid, the administration of [131]iodine radionuclide in HGCs dosage form is constrained to small doses. While further investigation is needed, this study provides a promising method for safely increasing the volume and consequently the dose administered, with the compliance of cancer patients receiving an oral radiotherapy regimen.

Conflict of Interest

The authors declare that they don't have any conflict of interest.

REFERENCES

1. Stegemann, S.; Bornem, C., *Hard gelatin capsules today-and tomorrow*. Capsugel Library: 1999.
2. Kamalakkannan, V.; Puratchikody, A.; Masilamani, K.; Senthilnathan, B. Solubility enhancement of poorly soluble drugs by solid dispersion technique–A review. *J. Pharm. Res.* **2010,** *3* (9), 2314-2321.
3. De Villiers, M. M. Oral Conventional Solid Dosage Forms: Powders and Granules, Tablets, Lozenges, and Capsules. *Theory and Practice of Contemporary Pharmaceutics. CRC Press LLC. New York* **2005**, 314-23.
4. Marques, M. R.; Cole, E.; Kruep, D.; Gray, V.; Murachanian, D.; Brown, W. E.; Giancaspro, G. In *Liquid-filled gelatin capsules*, Pharmacopeial forum, 2009; pp 1029-1041.
5. Zhang, X.; Do, M. D.; Casey, P.; Sulistio, A.; Qiao, G. G.; Lundin, L.; Lillford, P.; Kosaraju, S. Chemical cross-linking gelatin with natural phenolic compounds as studied by high-resolution NMR spectroscopy. *Biomacromolecules* **2010,** *11* (4), 1125-1132.
6. Bigi, A.; Panzavolta, S.; Rubini, K. Relationship between triple-helix content and mechanical properties of gelatin films. *Biomaterials* **2004,** *25* (25), 5675-5680.
7. de Moura, A. B.; del Mastro, L. Effect of electron beam irradiation on mechanical properties of gelatin/brazil nut shell fiber composites. *Revista Matéria* **2010,** *15* (2), 414-419.
8. Dorozhkin, S. V. Nanodimensional and nanocrystalline apatites and other calcium orthophosphates in biomedical engineering, biology and medicine. *Materials* **2009,** *2* (4), 1975-2045.
9. Safrany, A.; Miller, A.; Kovacs, A. Guidelines for the Development, Validation and Routine Control of Industrial Radiation Processes. International Atomic Energy Agency. IAEA, Publications, Sales and Promotion Unit: 2013.
10. Benson, R. S. Use of radiation in biomaterials science. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms* **2002,** *191* (1), 752-757.
11. Cilurzo, F.; Selmin, F.; Minghetti, P.; Montanari, L.; Lenardi, C.; Orsini, F.; Poletti, G., Comparison between gamma and beta irradiation effects on hydroxypropylmethylcellulose and gelatin hard capsules. *AAPS Pharm. Sci. Tech.* **2005,** *6* (4), E586-E593.
12. Jo, C.; Kang, H.; Lee, N. Y.; Kwon, J. H.; Byun, M. W., Pectin-and gelatin-based film: effect of gamma irradiation on the mechanical properties and biodegradation. *Radiat. Phys. Chem.* **2005,** *72* (6), 745-750.
13. del Mastro, N. L. Irradiation of gelatin. Important applications for the development of new materials. **2011**.
14. Zaman, H. U.; Beg, M.; Khan, M. A.; Khan, R. A., A comparative study of gamma and ultraviolet radiation on gelatin film with 2-ethylhexyl

acrylate. *J. Adhes. Sci. Tech.* **2013,** *27* (24), 2653-2665.

15. Perkasa, D. P.; Erizal, E.; Darmawan, D.; Rasyid, A., Effect of gamma irradiation on mechanical and thermal properties of fish gelatin film isolated from lates calcarifer scales. *Indones. J. Chem.* **2013,** *13* (1), 28-35.

16. Ofner, C. M.; Zhang, Y. E.; Jobeck, V. C.; Bowman, B. J. Crosslinking studies in gelatin capsules treated with formaldehyde and in capsules exposed to elevated temperature and humidity. *J. Pharm.Sci.* **2001,** *90* (1), 79-88.

17. Rockville, M. Disintegration and Dissolution of Dietary Supplements h2040i. In *USP. USP 31–NF 26*, 2008; Vol. US Pharmacopeial Convention; , pp 732–736.

18. Islam, M. M.; Zaman, A.; Islam, M. S.; Khan, M. A.; Rahman, M. M. Physico-chemical characteristics of gamma-irradiated gelatin. *Prog. Biomater.* **2014,** *3* (1), 21.

19. Kamińska, A.; Sionkowska, A. Effect of UV radiation on the infrared spectra of collagen. *Polym. Degrad. Stab.* **1996,** *51* (1), 19-26.

20. Cataldo, F.; Ursini, O.; Lilla, E.; Angelini, G., Radiation-induced crosslinking of collagen gelatin into a stable hydrogel. *J. Radioanal. Nucl. Chem.* **2007,** *275* (1), 125-131.

21. Digenis, G. A.; Gold, T. B.; Shah, V. P. Cross-linking of gelatin capsules and its relevance to their in vitro-in vivo performance. *J. Pharm. Sci.* **1994,** *83* (7), 915-21.

22. Chiwele, I.; Jones, B. E.; Podczeck, F. The shell dissolution of various empty hard capsules. *Chem. Pharm. bull.* **2000,** *48* (7), 951-956.

23. Honkanen, O.; Pia, L.; Janne, M.; Sari, E.; Raimo, T.; Martti, M. Bioavailability and in vitro oesophageal sticking tendency of hydroxypropyl methylcellulose capsule formulations and corresponding gelatin capsule formulations. *Eur. J. Pharm. Sci.* **2002,** *15* (5), 479-488.

24. Ku, M. S.; Li, W.; Dulin, W.; Donahue, F.; Cade, D.; Benameur, H.; Hutchison, K. Performance qualification of a new hypromellose capsule: Part I. Comparative evaluation of physical, mechanical and processability quality attributes of VCaps Plus®, Quali-V® and gelatin capsules. *Int. J. Pharm.* **2010,** *386* (1), 30-41.

25. Al-Tabakha, M. M. HPMC capsules: current status and future prospects. *J. Pharm. Pham. Sci.* **2010,** *13* (3), 428-442.

26. Sansone, M. A. Hydrodynamic Properties of Gelatin –Studies from Intrinsic Viscosity Measurements; *In: Johan Verbeek "Products and Applications of Biopolymers"* Chap. 5, pp.85-116, Pub. InTech, ISBN 978-953-51-0226-7. **2012**.

27. Gordon, P. W.; Brooker, A. D. M.; Chew, Y. M. J.; Wilson, D. I.; York, D. W. Studies into the swelling of gelatin films using a scanning fluid dynamic gauge. *Food Bioprod. Process* **2010,** *88* (4), 357-364.

28. Mercadé-Prieto, R.; Sahoo, P. K.; Falconer, R. J.; Paterson, W. R.; Ian Wilson, D. Polyelectrolyte screening effects on the dissolution of whey protein gels at high pH conditions. *Food Hydrocoll.* **2007,** *21* (8), 1275-1284.

29. Duconseille, A.; Astruc, T.; Quintana, N.; Meersman, F.; Sante-Lhoutellier, V. Gelatin structure and composition linked to hard capsule dissolution: a review. *Food Hydrocoll.* **2015,** *43*, 360-376.

30. Yang, X. J., Zheng, P. J., Cui, Z. D., Zhao, N. Q.,Wang, Y. F., & De Yao, K. Swelling behaviour and elastic properties of gelatin gels. *Poly. Inter.* **1997** *44,* 448-452.

31. Miyawaki, O.; Norimatsu, Y.; Kumagai, H.; Irimoto, Y.; Kumagai, H.; Sakurai, H. Effect of water potential on sol-gel transition and intermolecular interaction of gelatin near the transition temperature. *Biopolymers* **2003,** *70* (4), 482-491.