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Effect of γ-dose rate and total dose interrelation on the polymeric hydrogel: A novel injectable male contraceptive ☆

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ABSTRACT

Functional necessity to use a particular range of dose rate and total dose of γ-initiated polymerization to manufacture a novel polymeric hydrogel RISUG® (reversible inhibition of sperm under guidance) made of styrene maleic anhydride (SMA) dissolved in dimethyl sulphoxide (DMSO), for its broad biomedical application explores new dimension of research. The present work involves 16 irradiated samples. They were tested by fourier transform infrared spectroscopy, matrix assisted laser desorption/ionization–TOF, field emission scanning electron microscopy, high resolution transmission electron microscopy, etc. to see the interrelation effect of gamma dose rates (8.25, 17.29, 20.01 and 25.00 Gy/min) and four sets of doses (1.8, 2.0, 2.2 and 2.4 kGy) on the molecular weight, molecular weight distribution and porosity analysis of the biopolymeric drug RISUG®. The results of randomized experiment indicated that a range of 18–24 Gy/min γ-dose rate and 2.0–2.4 kGy γ-total doses is suitable for the desirable *in vivo* performance of the contraceptive copolymer.

1. Introduction

Although some medical devices and other functional biopolymer products have been manufactured with the help of gamma irradiation polymerization (Vakkalanka and Peppas, 1996; Dybek et al., 1992; Kaetsu, 1981; Singh et al., 1980; Rosiak and Yoshii, 1999; Peppas, 1986) there seems to exist a big lacunae when one thinks about the process control especially interrelated effect of dose rate–total dose combination on the specific functional characteristics and *in vivo* profile of the product (Lam et al., 2002), and the pieces of work in this field keeps the most important question unanswered that to which extent this particular process parameter goes to affect the product reproducibility (Zaikov and Sharpatyi, 2006), the key feature of any biopolymer development (Guha, 1996; Sharma et al., 2003; Guha, 2005).

Designing of polymeric materials has played an enormous role in the success of medical devices and drug delivery systems (Langer and Tirrell, 2004; Nair and Laurencin, 2006). Now the question arises here that how designing of materials can be controlled? Material designing for any product development depends on the control of the process parameters. Amongst all process parameters responsible for any product development/performance one has to distinguish the controllable and uncontrollable parameters (Ulanski et al., 2002). For controllable factors all options should be tried in advance with maximum possible variation for achieving desirable drug characteristics (McLaughin, 1977). For example, manufacturing of ocular implants took help of controlling dose rate and total dose (Goldberg et al., 1989).

Vital process parameters for γ-irradiation induced biopolymer styrene maleic anhydride (SMA) manufacturing being dealt with in the present research are dose rate, total dose and irradiation environment. Amid these most important factors are the dose rate that controls the rate of the reaction and the total dose that determines the cross-linking phenomenon of the polymer produced (Delides and Shepherd, 1977; Toy and DiBari, 1972). This scenario indicates that dose rate–total dose interrelation process parameter remains one of the most important controllable factors that directly affects biopolymer product quality as well as reproducibility. At laboratory scale dose rates and total doses are maintained and calibrated by dosimetry with the use of different chemicals and appropriate geometry of the lead shield (Gupta et al., 2000; FDA, 1970; NCRP, 2004).

Literature do not find good number of correlation studies on γ-irradiation dose rate and total dose interrelation with product performance but a good amount of work influenced individually by one of these parameters has been reported. Studies on the

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effect of γ-ray dose rate on polymerization, emulsion and microemulsion polymerization of styrene were initiated. At constant polymerization period while changing dose rate the polymerization rate and the molecular weight of the biopolymer varies (Xu et al., 2000; Hausberger et al., 1995). Relevant data demonstrated a substantial effect of γ-irradiation on initial molecular weight distribution and onset of mass loss for PLGA, but no effect on rate of mass loss. For a given absorbed dose, radiation-induced damage to a polymer in air environment usually depends on the dose rate of the exposure. The values of elongation at break and the thermo-oxidative stability decreases with the advanced degradation, density tends to increase with the absorbed dose (Plaek et al., 2003).

Irradiation parameter controlled relation provides better biological performance of polymer (Kausch and Anjum, 2003; Yagoubi et al., 1999; Yonetani and Grassley, 1970). For instance, chemical and physical properties of ultra-high-molecular-weight-polyethylene (UHMWPE) is modified by high absorption dose of ionizing radiations (Torrisi et al., 2007). γ-irradiation (5–100 kGy) of poly (lactide) microspheres reduced the average molecular weight of the polymer and increased the carboxylic acid content as a function of the dose (Yoshioka et al., 1995a). Molecular weights of polystyrene formed in methylene chloride are higher than those obtained in bulk polymerization in toluene, which suggests strong confirmation of a cationic mechanism in radiation induced polymerization (Ueno et al., 1966; Beach et al., 2003). Studies on the use of γ-irradiation as a method for controlling the drug release from poly (dl-lactide) microspheres (Yoshioka et al., 1995b; Montanari et al., 2003; Desai and Park, 2006) found that the time period before the start of rapid release could be controlled by altering the irradiation dose.

Present paper has considered molecular weight as an important characteristics varying with the changes in process parameters and that in turn directly affect biological performance of the drug. SMA having molecular weight in the range of 60–90 kDa is considered optimum for long-time contraception with desirable biological performance. In past, influence of molecular weight on the biological effects of Benzocaine modified maleic anhydride copolymer revealed that the maleic anhydride synthesized copolymers exhibit prolonged half-life serum, accumulate in K_{562} erythroleukemia solid tumors, and inhibit tumor growth (Constantin et al., 2006). During studies on biological effect, the product of chitosan irradiated at 100 kGy with a molecular weight of approximately 16 kDa showed the strongest growth promotion effect on plants *in vitro* (Luan et al., 2005). Other studies focused on finding the optimal γ-ray dose found to be 50 kGy and irradiation conditions for producing low-molecular-weight chitosan that retains its chemical structure (Yoksan and Akashi, 2004).

The biopolymer hydrogel RISUG® (acronym for Reversible Inhibition of Sperm Under Guidance) used in this study is currently undergoing extended Phase III clinical trials (Sharma et al., 2007) after successful completion of previous trials (Guha et al., 1993; Guha et al., 1997). RISUG® consists of styrene maleic anhydride (SMA) copolymer dissolved in dimethyl sulphoxide (DMSO), later acting as a vehicle solvent. SMA being one of the most important products of radiation technique, present study covers the issue that why a particular dose rate with a particular total dose is required for specific product development in order to have controlled contraceptive characteristics.

Therefore, this paper investigates the interrelated effect of γ-dose rates 8.25, 17.29, 20.01 and 25.00 Gy/min, in correlation with four sets of total doses 1.8, 2.0, 2.2 and 2.4 kGy as process control tools, on some physico-chemical characteristics of the RISUG®, namely its molecular weight, functional group density, porosity characteristics, surface topology and biological effects of drug like swelling, coagulation, plaque formation and stability inside the reproductive tube. Beside calibration, total 16 SMA samples were irradiated and tested for each dose/dose rate combination. 16 batches of SMA thus produced were observed by fourier transform infrared spectroscopy (FTIR), matrix assisted laser desorption/ionization–time of flight (MALDI–TOF) and field emission scanning electron microscopy (FESEM), respectively.

2. Experimental

2.1. Synthesis of biopolymer drug RISUG®

2.1.1. Gamma cell-5000 calibration

Dose rates have been determined by Fricke dosimetry (ASTM, 1997; Upadhyay et al., 2002; Klassen et al., 1999) where lead shield were used to diversify the dose rates. For gamma cell-5000 calibration (Gupta and Bhat, 1986) experiments were performed without lead shield in 10 fixed time intervals and with lead shields of three types at 10 fixed time intervals. Irradiation time (minutes) versus UV absorbance graph (Simanzadu 349) for Fricke solution irradiated without attenuation delivered a dose rate of 105 Gy/min whereas attenuation with lead shield of thicknesses 2.2×10^{-2}, 2.8×10^{-2} and 3.8×10^{-2} m produced dose rates 25.00, 17.29 and 8.25 Gy/min, respectively. Based on this calibration, SMA copolymer was produced.

2.1.2. Synthesis of RISUG®

Styrene and maleic anhydride monomer (Fluka), after rigorous purification, were taken in 1:1 ratio. Ethyl acetate (Glaxo) was added to the styrene and maleic anhydride mixture (1:1:7) and filtered N_2 gas purged into stopperred glass bottles for 5 min as per USP 5,488,075 (Guha, 1996). The polymerization was done for the required time in collaboration with lead shields of appropriate thickness in order to obtain required dose rate–total dose combination for each set of experiment (see Table 1). Short range of dose rate has been used for this study that goes well with the SMA copolymer synthesis for contraception. During polymerization other controllable irradiation factors were kept constant.

2.1.3. Post irradiation processing

Polymerization was followed by precipitation with petroleum ether (Merck) and soxhlet distillation using 1,2-dichloroethane and distilled water, respectively. Monomers were removed meticulously. The SMA obtained was purified, powdered and stored in stoppered sterile glass tubes. SMA powder compounded with DMSO (Sigma HYBRI-MAX sterile filtered USA) and transferred into specific type of prefilled syringe for injection.

Table 1
Experiment design of gamma irradiation polymerization to study dose rate–total dose interrelation effect where factors kept constant are monomer ratio (1:1), polymerization environment (N_2 and 25–30 °C) and Co-60 as irradiation source for gamma cell 5000.

Dose rate (Gy/min) Total dose (kGy)	C1:8.25	C2:17.29	C3:20.01	C4:25.00
R_1: 1.8	105 (M_1)	92 (M_5)	59 (M_9)	52 (M_{13})
R_2: 2.0	111 (M_2)	95 (M_6)	65 (M_{10})	57 (M_{14})
R_3: 2.2	119 (M_3)	98.5 (M_7)	72 (M_{11})	62 (M_{15})
R_4: 2.4	130 (M_4)	100.1(M_8)	81 (M_{11})	65(M_{16})

Unit of molecular weight (M1–M16)=kDa; R1, R2, R3 and R4 represent row 1, row 2, row 3 and row 4, respectively; and C1, C2, C3 and C4 stand for columns 1–4, respectively.

2.2. Characterization methods

2.2.1. Fourier transform infrared spectroscopy (FTIR)

Functional group variation analysis of every batch of finished SMA was done by FTIR (Thermo Nicolet Corporation). Solid powder samples for this study were milled with potassium bromide to form a very fine powder, which then compressed into a thin pellet for analysis. A set of four total doses 1.8, 2.0, 2.2, 2.4 kGy was kept constant with increasing dose rate in first half of experiment (represented by Figs. 1a–d) while in second half (represented by Figs. 1e–h) a set of four dose rates 8.25, 17.29, 20.01, 25.00 Gy/min was kept constant with increasing total dose.

2.2.2. Matrix assisted laser desorption ionization–time of flight (MALDI–TOF)

Mass spectra were recorded by a spectrometer model VOYAGER-DE^{TM} PRO, Applied Biosystems, Switzerland. Light from a pulsed nitrogen laser (VSL 337 ND, Laser Science Inc.), A=337 nm, 3 ns pulse, ~200 #J per pulse, is focused by a quartz lens onto the target. SMA powder samples for MALDI–TOF synthesized at different gamma dose rate and total dose combination followed by mixing with solvent DMSO (Sigma) in 1:100 ratios. Matrix 2, 5 dihydroxy benzoic acid (Sigma Aldrich) was mixed with DMSO (Sigma) in the ratio 1:100. Both solutions of SMA with DMSO and matrix with DMSO, respectively, were mixed together and spotted on stainless steel sample plates.

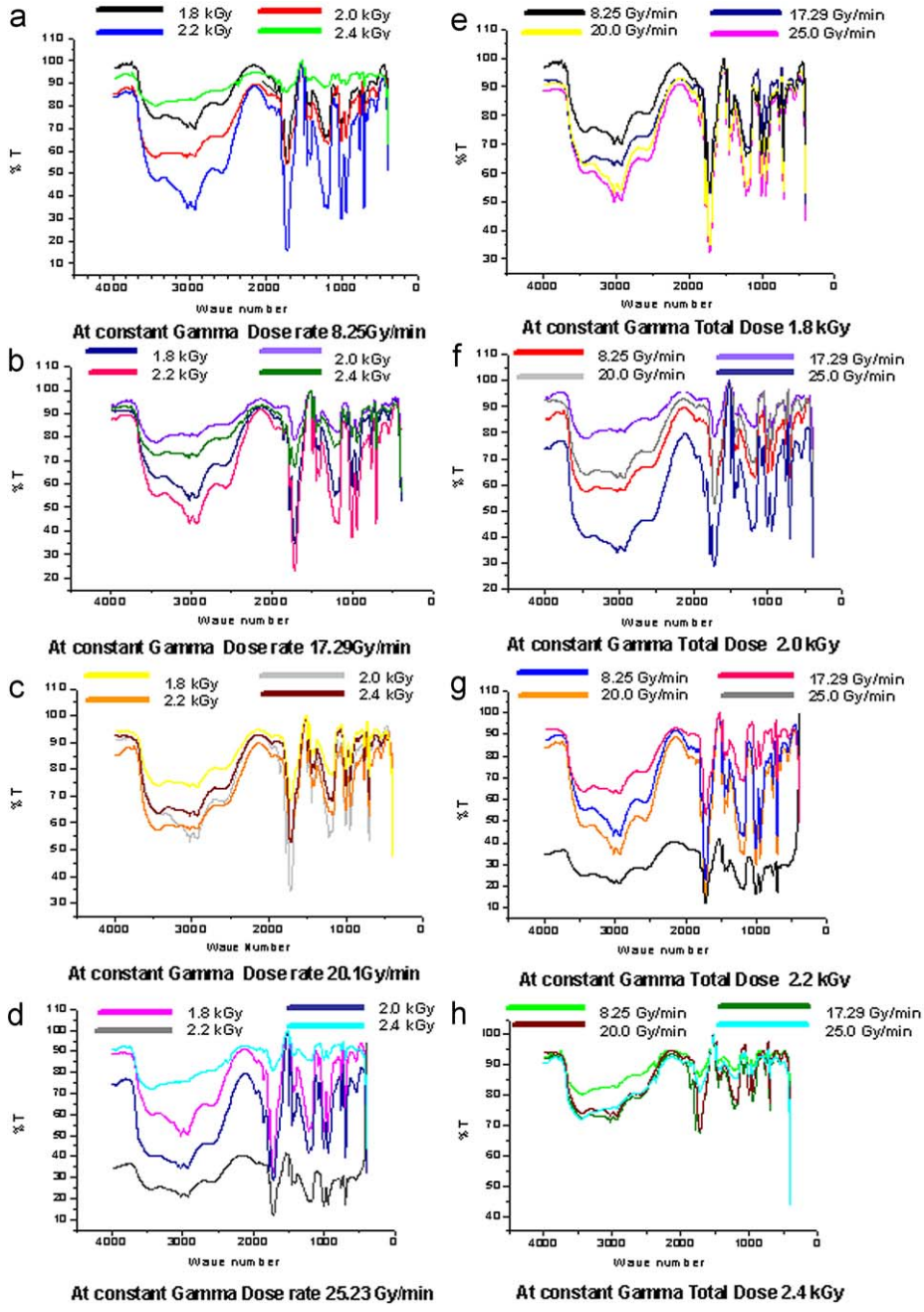

Fig. 1. Fourier transform infrared spectroscopy based comparative analysis for (a) constant dose rate of 8.25 Gy/min with varying total doses 1.8, 2.0, 2.2 and 2.4 kGy, (b) constant dose rate of 17.29 Gy/min with same varying total doses, (c) constant dose rate of 20.01 Gy/min with same varying total doses, (d) constant dose rate of 25.00 Gy/min with same varying total doses, (e) constant total dose 1.8 KGy with varying dose rates of 8.25, 17.29, 20.01 and 25.00 Gy/min, (f) constant total dose 2.0 kGy with same four varying dose rates, (g) constant total dose 2.2 kGy with similarly four varying dose rates and (h) constant total dose 2.4 kGy with similarly four varying dose rates (8.25, 17.29, 20.01 and 25.00 Gy/min).

Approximately 2 ml of the mixture was applied to a substrate and allowed to dry at ambient conditions. VOYAGER MS software was used for this analysis with plate magnification 600×. The principle of analysis lies in the total number of molecules having a particular range of molecular weight (low/high). Average number molecular weight (M_n) was calculated as per the following equation:

$$M_n = \sum Ii\, M_i / \sum I_i \quad (1)$$

Where M_n is the number average molecular weight, *Ii* is the integrated area under each peak and M_i*is* the chemical mass of the biopolymer.

2.2.3. Field emission scanning electron microscopy (FESEM)

For post drug implantation studies rat and rabbit subjects were used as per the Institute Animal Ethics Committee (I.A.E.C.) rules (Jha et al., 2009a, 2009b). All animals were reared at Animal House, Department of Biotechnology, Indian Institute of Technology, Kharagpur for this experiment before and after injection. *In vivo* profile of the SMA over a period of time was tested in animal subjects by injecting RISUG® into the reproductive tube of subjects (n=5 for each SMA sample). After 35 days the drug was recovered and observed under FESEM instrument model SUPRA™ 40, ZEISS, Germany provided with Carl Zeiss SMT–Nano Technology Systems Division. Samples for this microscopic analysis consist of thin films of recovered SMA. Scale on micrographs helped to assess the SMA porosity caused due to the drug coming in contact with the body fluid inside reproductive tube as per the following equation:

$$\text{Porosity} = 1 - \left(\frac{\text{Apparent molecular density}}{\text{Total density of compound}}\right) 100 \quad (2)$$

2.2.4. High resolution transmission electron microscopy (HRTEM)

The SMA powder and precipitated SMA films were both investigated by HRTEM using a JEM 2100, Oxford Instruments, Oxfordshire, UK. SMA precipitates were freshly prepared and dried before observation. The powder sample was ultrasonically dispersed in acetone to form very dilute suspensions and then the dilute sample was dropped on a copper grid with carbon coated film. The ultrathin samples were deposited on 1000-mesh copper grids covered with a carbon-coated perforated film to minimize background. A double-tilt specimen holder was used to tilt several zone axis.

3. Results

3.1. Absorption spectrum analysis

FTIR observation indicates that with increasing dose rate the peak intensity for individual SMA samples significantly varies between Figs. 1a and b but the variation is less significant across Figs. 1b–c and c–d, though it changes certainly. 1600–1900 cm^{-1} peak intensity is quite reasonable that represents anhydride formation. At low dose rate, the functional group status indicated by the peak intensity indicates that anhydride group do not change but other groups like aliphatic groups represented by carbon chain intensity is more abundant than anhydride functional group. With increasing dose rate carbon chain intensity decreases across Figs. 1a–c and Fig. 1c gives optimum value. Parallel to these changes, anhydride functional group represented by 1774 and 1857 cm^{-1} remains almost constant with optimum value in Fig. 1c, which indicates styrene maleic anhydride copolymer synthesis. At very high dose rate like 25.00 Gy/min, the decrease in intensity of functional group C-chain indicated decrease in cross-linking. This results in decrease of the average molecular weight with increasing dose rate (Figs. 1a–d).

When total dose increased from 1.8 (Fig. 1e) to 2.0 kGy (Fig. 1f), the molecular weight significantly increases due to increase in C-chain abundance. Similarly increase in total dose from 2.0 to 2.2 kGy lead to an increase in molecular weight across Figs. 1f–g but the change is less significant here. In summary, average molecular weight increased with increase in total dose (Figs. 1e–h).

3.2. Mass spectroscopy

Figs. 2a, b and 3a shows that with increase of dose rate from 8.25 to 25.00 Gy/min, M_n decreases due to average number of molecular mass moving towards lower side. For example Fig. 2a indicates M_n, 119 kDa at dose rate 8.25 Gy/min and total dose 2.2 kGy. In this case, the molecular weight distribution spreads from 70 to 130 kDa. On the other hand, in Fig. 2b molecular weight distribution spreads from 40 to 100 kDa where sample was irradiated at 20.01 Gy/min dose rate with 2.2 kGy total dose. Further Fig. 3a shows that molecular weight distribution range is 10–70 kDa at dose rate 25.00 Gy/min with total dose 2.2 kGy.

On the contrary, with increasing total dose 1.8–2.4 kGy at constant dose rate of 8.25 Gy/min the M_n of the compound increases from 105 to 130 kDa due to increase of molecular interaction time leading to slight increase in the molecular distribution shifting from lower side to higher side. The rate of molecular weight increment phenomenon, due to increase of total dose at constant dose rate, decreases as dose rate proceeds from 8.25 to 25.00 Gy/min which owes to increase of direct gamma ray interaction with the monomers (Fig. 3b).

3.2.1. Effect of dose rate on molecular weight distribution

The molecular weight range more than 92 kDa obtained for samples M_6, M_7 and M_8 is beyond optimum (Table 1). M_{16} observed the optimal molecular weight 65 kDa at dose rate 25.00 Gy/min with total dose 2.4 kGy combination. In general, in rows the molecular weight decreases with increase in dose rate at constant total dose from left to right; here the change in molecular weight is quite remarkable. From top to bottom C1 depicts constant low dose rate 8.25 Gy/min with increase in total dose resulting in increase of molecular weight though to lesser extent. Similar results found in C2, C3 and C4 with constant dose rates 17.29, 20.01 and 25.00 Gy/min, respectively, with increase in total dose resulting in not so significant increase of molecular weight. Molecular weight from left to right decreases due to increase of dose rate while it is increasing from top to bottom in columns due to increase of total dose.

3.3. Copolymer porosity effecting biological performance of drug

SMA copolymer porosity is directly proportional to the swelling of RISUG® in biological environment that in turn controls degree of blockage of the reproductive tube. As partial blockage is one of the key features desired for ideal RISUG® functionality, *in vivo* profile of the drug SMA was tested by injecting it into the reproductive tube. Post drug implantation swelling of the SMA sample irradiated at 8.25 Gy/min (Figs. 4a–d) and blockage caused in the reproductive tube due to its presence was most significant in comparison to implantation of drug samples irradiated at 17.29 Gy/min (Figs. 4e–h), 20.01 Gy/min (Figs. 4i–l) and 25.00 Gy/min (Figs. 4m–p). This indicates increase of dose rate caused decrease of swelling due to decreasing porosity characteristics. On the other hand, increasing the total dose upto 2.4 kGy while keeping the dose rate constant at

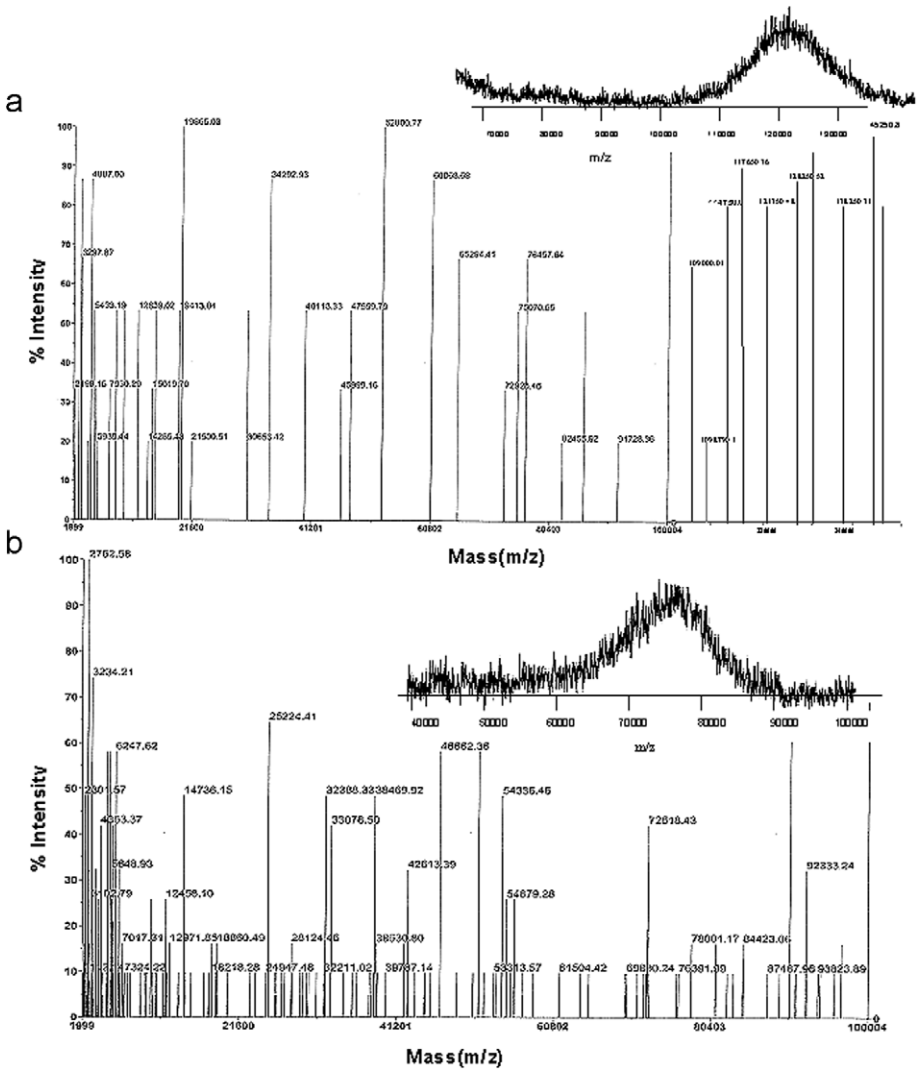

Fig. 2. MALDI–TOF based molecular weight analysis of SMA. (a) Molecular weight (M_n) of M_3 sample irradiated at dose rate 8.25 Gy/min with total dose 2.2 kGy is 119 kDa and distribution pattern ranges from 70 to 130 kDa. (b) Molecular weight (M_n) of M_{11} sample irradiated at dose rate 20.01 Gy/min with total dose 2.2 kGy is 72 kDa and distribution pattern ranges from 40 to 100 kDa.

8.25 Gy/min (Fig. 4d), observed maximum blockage confirmation. Similar was the trend seen when total dose was increased for different set of constant dose rate. Swelling of RISUG® post implantation due to porosity of raw material SMA suggests that Figs. 4e, i–l and p are acceptable as they neither block nor swapped away with the body fluid.

3.4. Topological analysis

The HRTEM micrographs taken near the SMA powder as well as precipitate film are depicted in Figs. 5a–h. As shown in Fig. 5a the compound is quite clustered at 8.25 Gy/min. Fig. 5b gives idea of reduced clustering whereas optimal condition was being observed in Fig. 5c in which SMA particles do not show any overlapping. The particle are sparsely arranged in SMA powder prepared at 25.00 Gy/min (Fig. 5d). Similarly in case of SMA precipitate, that of 8.25 Gy/min (Fig. 5e) observes significant clustering, drug precipitate of 17.29 Gy/min shows less clustering (Fig. 5f), that of 20.01 Gy/min again gives desirable kind of RISUG® precipitate where plaque like structure (Fig. 5g) is observed and Fig. 5h shows almost acceptable characteristics.

4. Discussion

Lead shield attenuation based calibration helped to achieve the desired dose rate and total dose for each of total 16 rounds of SMA manufacturing experiment on laboratory scale. SMA synthesized in every batch of dose rate/total dose has its characteristics physico-chemical property which in turn directly affected biological performance of the drug RISUG® when injected into the reproductive tube of the animal subjects.

On correlation with MALDI–TOF observations the framework illustrated in Table 1 acknowledged that the biopolymer SMA being used for RISUG® synthesis is gamma dose rate sensitive. At constant dose rate 25.00 Gy/min, effect of total dose increment from 1.8 to 2.4 kGy on molecular weight distribution of biopolymer is 50% less compared to molecular weight distribution change in the range of total dose 1.8–2.4 kGy at constant dose rate 8.25 Gy/min. With dose rate increment from 8.25 to 25.00 Gy/min, effect of changes in total dose gave optimal change of molecular weight distribution which is more suitable for biological environment. Dose rate–total dose interrelation of gamma ray has significant effect on molecular weight change pattern, which leads to development of more suitable radiation biomaterial being used as male contraception device.

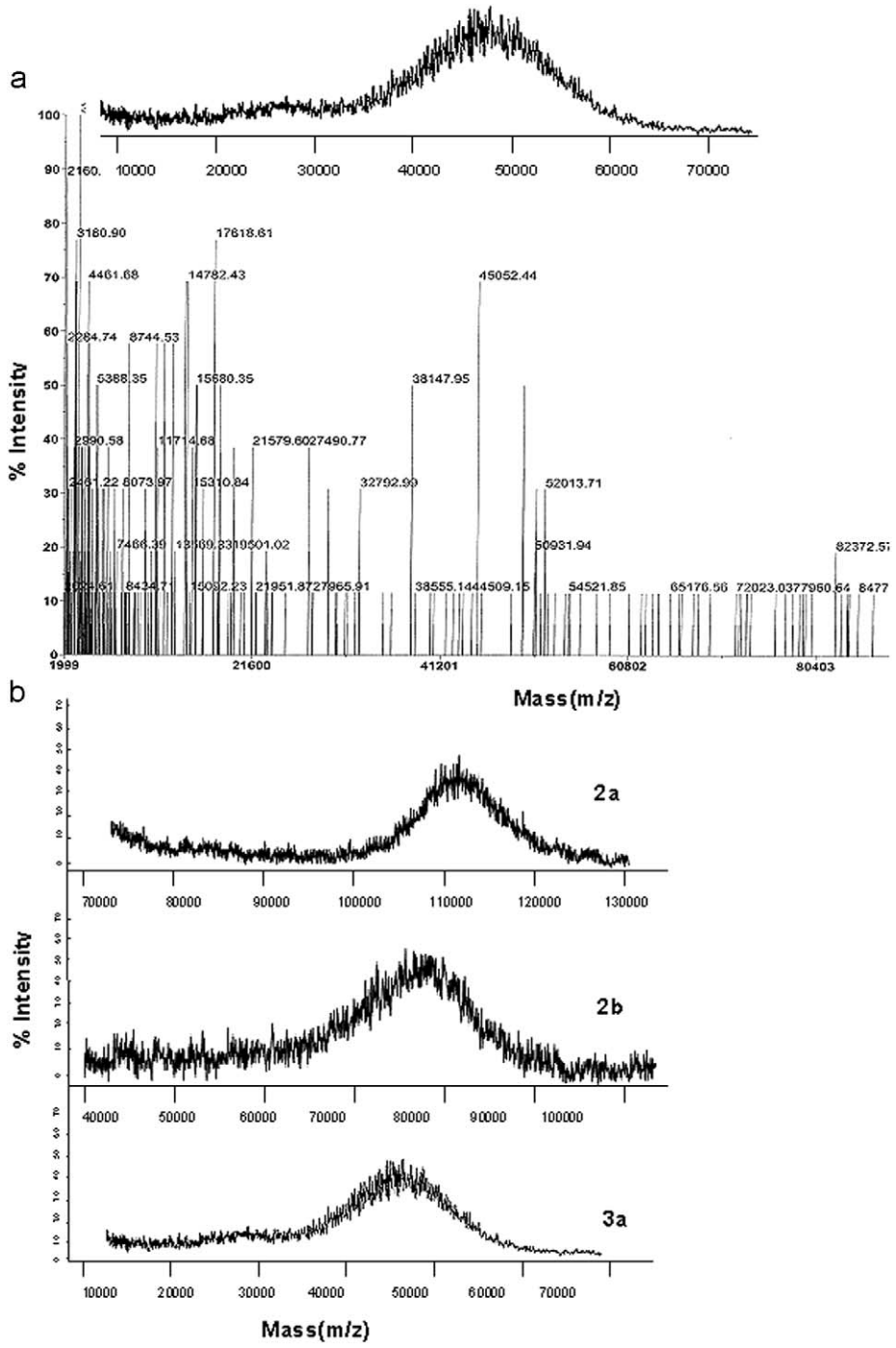

Fig. 3. MALDI–TOF based molecular weight distribution pattern study. (a) Molecular weight (M_n) of M_3 sample irradiated at dose rate 25.00 Gy/min with total dose 2.2 kGy is 62 kDa whereas its distribution pattern ranges from 10 to 70 kDa, (b) illustrates that how dose rate increment shifts the molecular weight distribution range from higher molecular side to lower molecular side. This observation also explains that the increase of dose rate from 8.25 to 25.00 Gy/min (2a < 2b < 3a) decreases the rate of molecular weight increment (C1 > C2 > C3 > C4) due to increase of total dose from 1.8 to 2.4 kGy at constant dose rates.

On moving from right column to left in Table 1, SMA samples M_{13}, M_{14} and M_{15} (Figs. 4m–o) obtained before sample M_{16} of C4 and SMA samples M_1, M_2, M_3, M_4, M_6, M_7, M_8 of C1 and C2 (Figs. 4a–d and f–h) obtained after M_5 sample of C2 were not acceptable due to increase in porosity post drug implantation observed with FESEM. This biological performance of RISUG® can be attributed to the fact that the molecular weight range of about 60–90 kDa is acceptable for SMA compounds. Polymer surface porosity analysed by FESEM micrography indicated that the polymer is becoming mechanically interlocked within the substrate surface and remains embedded in the pores throughout.

The porosity characteristics of polymeric material is acceptable for M_5, whereas above that in samples M_6, M_7 and M_8 the material porosity is decreasing significantly, which caused the vas deference blockage. Similarly, it was observed that the porosity of polymeric material is acceptable for M_{16} while below that for samples M_{13}, M_{14} and M_{15} porosity of polymeric material decreased significantly, which caused instability of biopolymer to be retained inside the vas deference.

The basis for RISUG® efficacy is charge based as the carboxylic anion and protons produced in the vas lumen in contact with the vas fluid from a plaque sort of structure and also lowers the pH

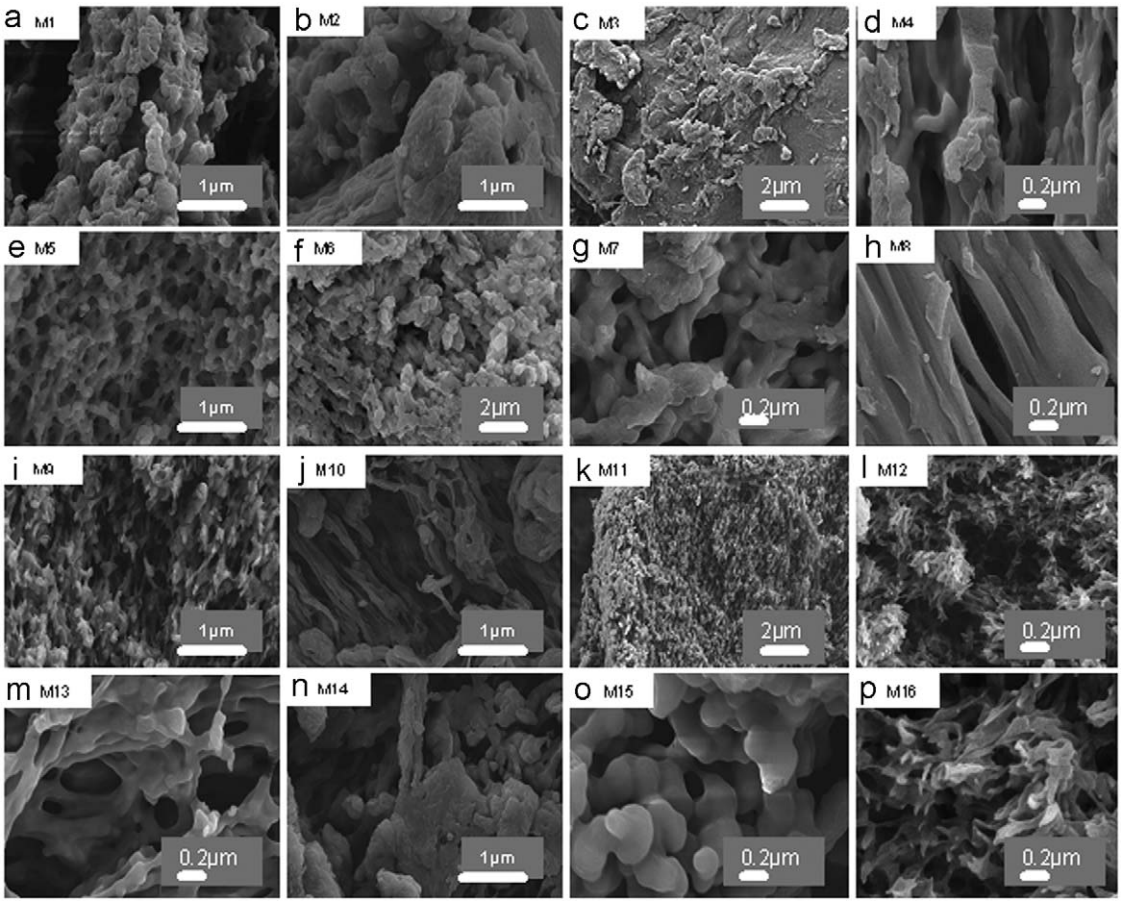

Fig. 4. Field emission micrography (FESEM) of drug recovered post implantation that was irradiated, respectively, at (a) 8.25 Gy/min, 1.8 kGy shows much significant swelling of the precipitate, (b) 8.25 Gy/min, 2.0 kGy illustrates comparatively less swelling (c) 8.25 Gy/min, 2.2 kGy (d) 8.25 Gy/min, 2.4 kGy also shows particle arrangement causing blockage (e) 17.29 Gy/min, 1.8 kGy (f) 17.29 Gy/min, 2.0 kGy irradiated drug also depicts significant swelling characteristics (g) 17.29 Gy/min, 2.2 kGy (h) 17.29 Gy/min, 2.4 kGy not acceptable due to its non uniform nature (i) 20.01 Gy/min, 1.8 kGy (j) 20.01 Gy/min, 2.0 kGy illustrates drug characteristics with desired level of swelling (k) 20.01 Gy/min, 2.2 kGy (l) 20.01 Gy/min, 2.2 kGy (m) 25.00 Gy/min, 1.8 kGy shows much porosity (n) 25.00 Gy/min, 2.0 kGy (o) 25.00 Gy/min, 2.2 kGy (p) 25.00 Gy/min, 2.4 kGy.

inside the vas tube (Guha et al., 1985; Guha, 2007).The contraceptive has been proved to be safe (Manivannan et al., 2005), antimicrobial (Sharma et al., 2003; Guha, 2005), long-term effective (Chaudhury et al., 2002; Jha et al., 2009a) and reversible (Lohiya et al., 2000). Non-invasive reversibility of SMA analogues under guidance of electromagnetic field is also possible (Jha et al., 2009b).

FTIR analysis illustrated that at low dose rate molecular weight is more due to C-chain abundancy that may completely block the vas deferens rendering flow of biological fluid difficult when copolymer synthesized in this case would be used as a fertility control agent. From Figs. 1e–h, increasing total dose resulted in less functional group intensity in Fig. 1h in comparison to Fig. 1e but the change is less remarkable than that for varying dose rate.

Overall, it is inferred that the effect of change in dose rate is more significant than the effect of change in total dose. Changes in dose rate in turn affects structural confirmation of the compound, and *in vitro* as well as *in vivo* performance of the drug (Jonathan, 2000). Actually, low dose rate gives high molecular weight that may block the reproductive tube. This in turn will cause several medical problems like sperm granuloma, hematoma, etc. Contrarily, at high dose rate the molecular weight is less due to decreased C-chain density and the compound would have lesser stability that may be swapped away with the body fluid giving threat to long-time stability and efficacy. In case of total dose a very short range (1.8–2.4 kGy), among which 2.0 and 2.4 kGy is most desirable one, has been used for SMA synthesis that serves the purpose of manufacturing contraceptive biopolymer with desired functionality.

MALDI–TOF analyses (Huang and Chandramouli, 2003) of total 16 samples explained that when dose total was switched to 2.2 from 2.0 kGy at 25.00 Gy/min, small increase in the molecular weight took place. The highest dose rate–total dose combination (25.00 Gy/min and 2.4 kGy) used for this experiment resulted in optimum molecular weight 65 kDa. This means on increasing the dose rate upto 1.2 Gy/min from optimum range of 18–24 Gy/min used for the synthesis of drug RISUG®, one may get desirable molecular weight inspite of very high or very low dose rate. Molecular weight analysis with laser method might have led to molecular structure deformation in some cases so help of field emission scanning electron microscopy (FESEM) was taken further for *in vivo* biological effect analysis (Furuno- Fukushi, 1996) with the same set of samples to see drug porosity regulating *in vivo* performance discussed previously.

As FESEM study crossed the dose rate of 17.29 Gy/min and went upto 25.00 Gy/min it was indicated that dose rate is a value that requires tolerance of 1–3 Gy/min. That means, although 18–24 Gy/min is optimum dose rate range, dosimetry variation and continuous source decay suggests that 17.46–24.6 Gy/min can very well be used for the SMA compound synthesis. The results obtained from FTIR, MALDI and FESEM got further confirmation with HRTEM results and it gives final saying about

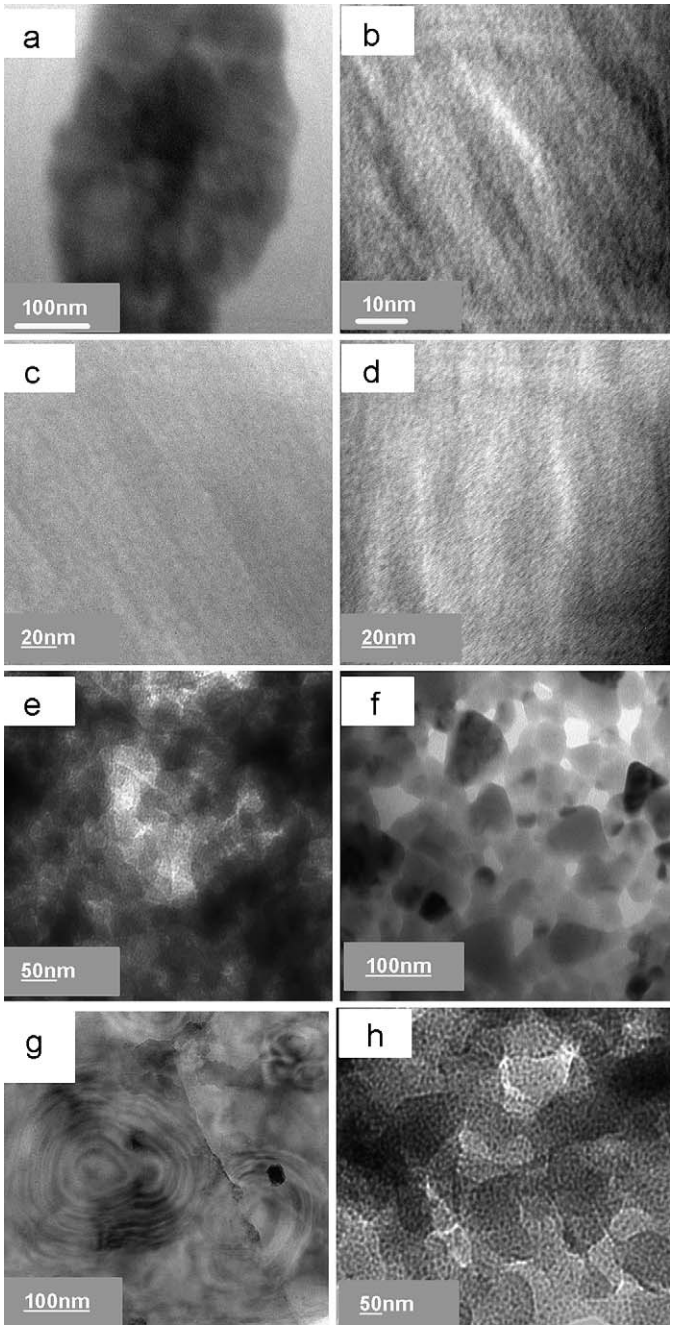

Fig. 5. HRTEM of SMA powder irradiated at (a) 8.25 Gy/min shows clustering (b) 17.29 Gy/min with much reduced clustering (c) 20.01 Gy/min showing optimum characteristics. (d) 25.00 Gy/min is near to desired morphology. RISUG® precipitates (e) 8.25 Gy/min with coagulated appearance (f) 17.29 Gy/min show less coagulation. (g) 20.01 Gy/min illustrates ideal plaque like structure. (h) 25.00 Gy/min may also be accepted.

what kind of compound is required that can be used as a novel male injectable contraceptive.

It is known that homogenous biopolymer product is formed only at medium high dose rate (Huglin and Zakaria, 1984). The compound SMA seen in Figs. 5a and e was not acceptable because at very low dose rate 8.25 Gy/min the molecular weight becomes quite high causing compound coagulation and structural deformation. On the other hand, at very high dose rate of 25.00 Gy/min, compound molecular weight becomes quite low causing sparsely arranged molecules in the compound (Figs. 5d and h) giving the compound a completely distorted shape and it becomes difficult to implant such drug in any one part of the reproductive tube as there always remains chances of such precipitate getting swapped away with the body fluid. SMA powder irradiated at 17.29 Gy/min (Fig. 5c) and precipitate (Fig. 5g) generated out of it appears to be less coagulated but this also cannot be accepted for *in vivo* application. But the polymer powder as well as precipitate of 20.01 Gy/min gives optimum morphological character that again suggests that 18–24 Gy/min dose rate at 2.0–2.4 kGy total dose being used for RISUG® manufacturing is the optimum condition and the plaque like structural arrangement is the most vital characteristics desired for implantation of the contraceptive RISUG® in the reproductive tube.

5. Conclusion

Present paper found indispensable role of dose rate–total dose interrelation for manufacturing of RISUG® biopolymeric compound while taking it from laboratory scale to manufacturing scale and states that extra effort (pre-irradiation calibration) is required to keep the dose rate constant with help of time to time calibration as the gamma ray source decays constantly. Among dose rate and total dose, dose rate has more significant impact in comparison to total dose in regulating biopolymer characteristics. Also, it should be kept in record that although optimum total dose is 2.4 kGy being used for RISUG® compound synthesis but dosimetry asks for a tolerance of 1–3 Gy/min in optimum dose rate range of 18–24 Gy/min. The manufacturing process parameter, dose rate–total dose interrelation taken as experimental tool affects the final compound and its physicochemical properties like molecular weight, mass distribution, porosity and topology which in turn affect the *in vivo* profile of the drug post implantation inside the reproductive tube. Thus it can be said that dose rate–total dose interrelation plays the most vital role in irradiation based polymeric drug manufacturing and its acceptability for biological use thereof.

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