Carbohydrate Polymers 90 (2012) 1685–1694

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Irradiation depolymerized guar gum as partial replacement of gum Arabic for microencapsulation of mint oil

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ARTICLE INFO

Article history:
Received 25 April 2012
Received in revised form 19 July 2012
Accepted 21 July 2012
Available online 27 July 2012

Keywords:
Guar gum
Irradiation
Mint oil
Microencapsulation
Storage stability

ABSTRACT

Spray dried microcapsules of mint oil were prepared using gum Arabic alone and its blends with radiation or enzymatically depolymerized guar gum as wall materials. Microcapsules were evaluated for retention of mint oil during 8-week storage during which qualitative changes in encapsulated mint oil was monitored using principal component analysis. The microcapsules with radiation depolymerized guar gum as wall material component could better retain major mint oil compounds such as menthol and isomenthol. The $t_{1/2}$ calculated for mint oil in microcapsules of gum Arabic, gum Arabic:radiation depolymerized guar gum (90:10), gum Arabic:enzyme depolymerized guar gum (90:10) was 25.66, 38.50, and 17.11 weeks, respectively. The results suggested a combination of radiation depolymerized guar gum and gum Arabic to show better retention of encapsulated flavour than gum Arabic alone as wall material.

1. Introduction

Microencapsulation of essential oil in carrier matrices is of great importance and relevance in the food and flavour industries by virtue of their ability to protect sensitive food components such as essential oils against the degradation reactions and loss of volatiles (Bringas-Lantigua, Expósito-Molina, Reineccius, López-Hernández, & Pino, 2011; Kadam, Hashmi, & Kale, 2011). Among the many techniques available for microencapsulation, spray drying is most preferred and commercially used. The extent of protection is evaluated on the basis of rate of loss of encapsulate from the microcapsules. Selection of an appropriate wall material is therefore crucial in microencapsulation by spray drying. Gum Arabic is the most frequently used wall material in the industry for encapsulation. However its high cost, inconsistent supply and varying quality has prompted investigations on alternative carrier materials (Kshirsagar & Singhal, 2007). Besides gum Arabic, use of carbohydrates such as maltodextrins and emulsifying starches (Reineccius, 1988, 1989) and protein matrices such as zein (Quispe-Condori, Saldaña, & Temelli, 2011), sodium caseinate, hydrolyzed casein (Drusch et al., 2012) and barley protien (Wang, Tian, & Chen, 2011) are previously reported.

Guar gum is a neutral polysaccharide consisting of β-D-mannose backbone with side chains of α (1→6) linked galactose residues obtained from endosperm of guar seeds. The ratio of mannose to galactose residues is approximately 2:1. India is the largest producer of guar gum and accounts for almost 80% of the global production (www.guargum.biz). The solution properties of guar gum depend primarily on its molecular weight (Jumel, Harding, & Mitchell, 1996). Aqueous solutions of guar gum have a very high viscosity even at low concentration which limits its application as a wall material for encapsulation. Hence, it needs to be depolymerized to lower molecular weight fractions for potential applications in flavour encapsulation.

Depolymerization of guar gum has been widely studied by several researchers. Enzymatic depolymerization using specific enzymes such as mannosidase and/or galactosidase (Mahammad, Prud'homme, Roberts, & Khan, 2006) is the most widely used method. Partially hydrolyzed guar gum (PHGG) is produced by controlled partial enzymatic hydrolysis of guar gum by β-endo-mannase. PHGG has a lower molecular weight around 20,000 Da and less viscosity than native guar gum. It is widely used as soluble dietary fibre and considered as GRAS (generally recognized as safe) since 1974 (Yoon, Chu, & Juneja, 2008).

In recent years, γ-irradiation induced depolymerization of guar gum has been reported (Dogan, Kayacier, & Ic, 2007; Gupta, Shah, Sanyal, Variyar, & Sharma, 2009; Jumel et al., 1996). γ-Irradiation is a physical process in which electromagnetic γ-rays or electron beam are directly exposed to food items for enhancing their safety and shelf life. The irradiation technology is approved by

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http://dx.doi.org/10.1016/j.carbpol.2012.07.051

FAO/IEAE/WHO joint committee on wholesomeness of food, and currently this technology is commercially practiced in several countries (Lacroix & Ouattara, 2000). Jumel et al. (1996) reported the molar mass and viscosity of the irradiated guar gum to decrease with an increase in irradiation dose without affecting its conformation.

Mint is used in large quantities in food, pharmaceutical and cosmetic industries. The global production of mint essential oil is estimated to be around 20,000 tonnes. India fulfils 80% of the total global demand with production of 16,000 tonnes of mint oil (Khanuja, 2007). Mint oil is a complex mixture of comparatively volatile and labile components such as β-pinene, limonene, methyl acetate, 3-octanol, isomenthone (Duriyaprapan & Britten, 1982). These components degrade during processing and storage which in turn alter the sensory properties of mint oil, making it difficult to manufacture and preserve products containing mint oil. Moreover, the poor solubility of mint oil in water is a constraint in the formation of its stable dispersion. Microencapsulation is the best option to enhance the stability of products formulated with mint oil (Jackson & Lee, 1991; Zhong et al., 2009). Soottitantawat et al. (2005) studied microencapsulation of L-menthol by spray drying using gum Arabic and modified starch (CAPSUL, HI-CAP 100). We have earlier reported on the development of hydrolyzed guar gum esters using oleic acid and octenyl succinic anhydride as alternative for gum Arabic for encapsulation, and evaluated their emulsion properties with the mint oil (Sarkar & Singhal, 2011).

To the best of our knowledge, there are no reports on flavour encapsulation using depolymerized guar gum as wall material. In the present study, an attempt has been made to use depolymerized guar gum as a partial substitute of gum Arabic for microencapsulation. Mint oil was microencapsulated using blends of radiation or enzyme depolymerized guar gum and gum Arabic as wall materials. The microcapsules were stored at 27 ± 2 °C for 8 weeks, and the retention of mint oil in the microcapsules was subsequently evaluated. Qualitative changes in mint oil during storage were evaluated using principal component analysis (PCA). PCA is a common processing method for analyzing unprocessed analytical data (Eriksson, Johansson, Kettaneh-Wold, & Wold, 2001).

2. Materials and methods

2.1. Materials

Guar gum and enzymatically prepared partially hydrolyzed guar gum (PHGG) were commercial samples manufactured and gifted by Lucid Colloids Ltd., Mumbai, India. Gum Arabic (Encapcia) was gifted by Colloides Naturels International (CNI), France. Mint oil was a gift sample from A.M. Todd, Mumbai, India. 2-Octanol (Purity 97.8%) was procured from Sigma Aldrich, Steinheim, Germany. Diethyl ether was procured from Merck India Ltd. and was distilled twice before use.

2.2. Methods

2.2.1. Preparation of guar gums for irradiation

Guar gum was purified before using it in the present study as described by Cunha, Vieira, Arriaga, De Paula, and Feitosa (2009) with some modifications. Briefly, guar gum (2% (w/v)) solution was dispersed in water by mixing with high speed mixer (Omnimixer, SORVALL, USA) for 5 min. The solution was kept overnight for complete hydration and then centrifuged (Beckman J2-MC, USA, Rotor JA 20) at 10,000 × *g* for 20 min. The supernatant was collected and the guar gum was precipitated by absolute alcohol. The precipitate so obtained was freeze dried (Christ, Beta 1-8 LD plus, UK with Preiffer vacuum pump, temperature −60 °C, vacuum 0.05 bar) and stored in air tight bottles until further use.

2.2.2. Irradiation of guar gum

In powder form, guar gum samples were irradiated at doses of 5, 10, 25, 50 and 100 kGy while in aqueous solution form (2% (w/v)) radiation doses were 1, 2, 3, 4, 5 and 6 kGy at a fixed dose rate of 4.438 kGy/h from a ^{60}Co gamma irradiator (GC-5000, BRIT, India). Gamma irradiator was calibrated by Fricke's dosimeter and dose uniformity ratio was 1.3. Guar gum was recovered from their irradiated aqueous solutions by precipitating with absolute alcohol. Precipitate was then freeze-dried and used for further studies.

2.2.3. Determination of molecular weight, polydispersity index and viscosity

Number average molecular weight (M_n), weight average molecular weight (M_w) and polydispersity index (PDI) (M_w/M_n) were determined by gel permeation chromatography (GPC) using a size exclusion column (5u, Biobasic SEC-1000, 300 mm × 4.6 mm, Thermo scientific, UK). A HPLC system (Ultimate 3000, Dionex corporation, Germany) equipped with a differential refractive index detector (RI-101, Shodex corporation, USA) was used. The mobile phase was deionized water (Millipore, Bedford, MA) with a fixed flow rate at 0.6 mL/min. All guar gum samples were injected (20 μL) as their aqueous solutions at concentrations of 0.01% (w/v). The sample solutions were centrifuged (12,000 rpm, 15 min) and filtered through 0.45 μm filter (Millipore, UK) prior to analysis. The column was calibrated using pullulan standards ranging from molecular weights of 342–2.56 million Da (Pullulan Standard, Fluka Analytical, St. Louis, USA). Pullulan standards were injected under the same HPLC conditions as described above.

Number average molecular weight (M_n) was calculated by the following equation:

$$M_n = \sum \left(\frac{N_i}{\sum N_i} \times M_i \right) \quad (1)$$

where N_i is the detector response at a particular time, $\sum N_i$ is the total detector response, and M_i is the molecular weight at given time.

Weight average molecular weight was calculated by following equation:

$$M_w = \sum \left(\frac{A_i \times M_i}{\sum A_i} \right) \quad (2)$$

where $A_i = N_i \times M_i$.

Based on M_n and M_w, PDI was calculated using equation given below

$$\mathrm{PDI} = \frac{M_w}{M_n} \quad (3)$$

For viscosity measurements, guar gum solution (2% (w/v)) was prepared and viscosity was measured by Brookfield viscometer DV1, spindle no. 4, 20 rpm, 27 ± 2 °C.

2.2.4. Preparation of mint oil emulsions

Twenty grams of gum Arabic alone or gum Arabic mixture with either of guar gum irradiated in solution form (IRS), guar gum irradiated in powder form (IRP) or enzymatically prepared partially hydrolyzed guar gum (PHGG) in proportions of 90:10 and 80:20 gum Arabic:guar gum was added in 100 ml of water and mixed on magnetic stirrer (500 rpm, 4 h). The solution so obtained was kept overnight at 27 ± 2 °C for proper hydration of polymers. Mint essential oil (3 g, 15% of wall material) was added and emulsion was prepared by shear mixing for 10 min at 3000 rpm using shear homogenizer (Indofrench Industries Engineers, Mumbai, Model

type-SPM-9) until complete dispersion of oil. Emulsion was stored at 4 °C for 24 h for complete stabilization of the oil–water interface.

2.2.5. Analysis of emulsions for emulsion stability index, turbidity, viscosity and particle size

A sample of liquid emulsion was transferred to a 10 mL measuring cylinder which was then capped and stored for 24 h. The volume of oil separated from the emulsion was measured from each emulsion stability index (ESI) within a possible range from 0 to 1 was recorded using Eq. (4) (Sarkar & Singhal, 2011).

$$\text{ESI} = 1 - \frac{\text{Total volume of separated oil from emulsion}}{\text{Total volume of oil in emulsion}} \quad (4)$$

A value of 0 represents poor emulsion stability, while a value of 1 represents high emulsion stability. The stability of the emulsions was further determined by measuring its turbidity (Pearce & Kinsella, 1978). A 10 μL aliquot of the emulsion was diluted to 1 mL with water and the absorbance (A) was measured at 650 nm using a U-2001 spectrophotometer (Hitachi) in a 1 cm path length cell. Emulsions were prepared in triplicate to check its reproducibility. Eq. (5) given below shows the relation between turbidity (τ) and absorbance (A) at 630 nm.

$$\tau = \frac{2.303 \times A}{l} \quad (5)$$

Viscosity of the emulsions was measured using Brookefield viscometer (Stoughton, MA, USA) at 27 ±2 °C with a sample volume of 65 mL and using LV-2 spindle, operated at 60 rpm.

BioVis Image analyzer plus (Expert Vision Labs Pvt Ltd., Mumbai, India) was used for determining the particle size of dispersed oil in emulsion at 0 h and after 24 h. A smear of the emulsion was prepared and observed under compound microscope. The digital camera associated with this instrument was used to take 10 photographs of the emulsion. Particle size of dispersed oil in emulsion was determined with the help of image analyzing software. Emulsion was analyzed in terms of particle size distribution, maximum, minimum and mean aspect (spheroidal), and minor and major axes (elliptical) of the particles. In this study, the mean aspect of the particle was considered, since the particles were spheroidal.

2.2.6. Preparation of microcapsules by spray drying

The stable emulsions (as indicated in Section 2.2.4) were spray dried (JISL, LSD-48 mini spray drier, Mumbai, India, inside chamber dimension: 100 cm height and 60 cm diameter) equipped with 0.5 mm diameter nozzle. The pressure of compressed air for the flow of the spray was adjusted to 2 bar. The inlet air temperature was maintained at 160 °C. A peristaltic pump was used to feed the spray drier at 400 mL/h. The microcapsules so prepared were collected from the collecting chamber and filled in airtight, self-sealable polyethylene pouches. Theses pouches were stored in a desiccator until further studies.

2.2.7. Analysis of microencapsulated mint oil

2.2.7.1. Determination of total and surface oil. The total oil content in the spray-dried microcapsules was determined. Briefly, encapsulated mint oil was extracted from 100 mg of encapsulated powder by steam distillation for 1 h in Nickerson apparatus (Nickerson & Likens, 1966). Double distilled diethyl ether was used as the extracting solvent. 2-Octanol was used as an internal standard for quantification of the oil. Each extract was dried over anhydrous sodium sulphate and concentrated by Kuderna Danish apparatus at (40 ± 0.05 °C) to a final volume of 500 μL by gentle steam of nitrogen.

Surface oil indicates the oil which is not contained within the microcapsules. It was estimated according to method described by Mortenson and Reineccius (2008) with minor modifications. The microencapsulated powder (500 mg) was extracted with 20 mL diethyl ether in a 50 mL conical flask and capped. 2-Octanol was used as an internal standard for quantification of the oil. The sample was put on an orbital shaker set at 150 rpm for 1 h at 27 ± 2 °C. The sample was then allowed to stand for settling of the powder. It was then filtered through a filter paper (Whatman no. 1) and the filtrate was concentrated by gentle steam of nitrogen. The composition of the total and surface oils was analyzed by gas chromatography (Shimadzu Corporation, Kyoto, Japan) provided with a micro thermal conductivity detector and equipped with a RTX5 (Restek Corporation, USA) capillary column with 30 m length; 0.25 mm I.D. and film thickness of 0.25 μm. The operating conditions were an initial column temperature of 40 °C with hold time of 5 min. Column temperature was then raised to 200 °C at the rate of 4 °C/min with hold time of for 2 min and further to 280 °C at the rate of 10 °C/min holding for 10 min; injector and interface were kept at 210 and 280 °C, respectively. Helium was used as carrier gas with flow rate of 1.5 mL/min. Temperature of TCD was 290 °C with current value set at 75 mA. All samples were analyzed in triplicates.

Mint oil obtained from steam distillation was analyzed using GC–MS (Shimadzu Corporation, Kyoto, Japan) having GC-17A gas chromatograph provided with DB-5 (J&W Scientific, CA, USA) capillary column (30 m length; 0.25 mm I.D. and 0.25 μm film thickness). The operating conditions were column temperature programmed from 60 to 200 °C at the rate of 4 °C/min, held at initial temperature and at 200 °C for 5 min, and further to 280 °C at the rate of 10 °C/min, held at final temperature for 20 min. Injector and interface temperatures were 210 and 280 °C, respectively. Helium (flow rate, 1.5 mL/min) was used as carrier gas. MS parameters were 70 eV ionization voltage and electron multiplier voltage of 1 kV. Peaks were tentatively identified by comparing its mass fragmentation pattern with standard spectra available in the spectral library (Wiley/NIST Libraries) of the instrument and with Kovats index.

2.2.7.2. Determination of encapsulation and entrapment efficiency. Encapsulation efficiency indicates the efficiency of the process to encapsulate and was determined as per Bule, Singhal, and Kennedy (2010). It was calculated as the ratio of the mass of mint oil present in the microcapsules to the mass of mint oil added at the time of emulsion preparation prior to spray drying. The encapsulation efficiency was calculated using Eq. (6).

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Total mint oil by experimental determination (g/gpowder)}}{\text{Therotical loading of mint oil (g/gpowder)}} \times 100 \quad (6)$$

The entrapment efficiency is defined as percent of oil entrapped in capsules and was calculated by Eq. (7)

$$\text{Entrapment efficiency (\%)} = \frac{\text{Total mint oil (g/gpowder)} - \text{surface mint oil (g/gpowder)}}{\text{Total mint oil (g/gpowder)}} \times 100 \quad (7)$$

2.2.8. Storage stability of mint oil microcapsules

Mint oil microcapsules obtained with gum Arabic, gum Arabic:IRS (90:10), gum Arabic:IRP (90:10) and gum Arabic:PHGG (90:10) as wall materials were analyzed for the content of total mint oil during 8-week storage at 27 ± 2 °C. A semi-log graph of percent retention of mint oil vs. time was plotted to obtain the rate constant (k) as the slope of the graph from which the half-life ($t_{1/2}$)

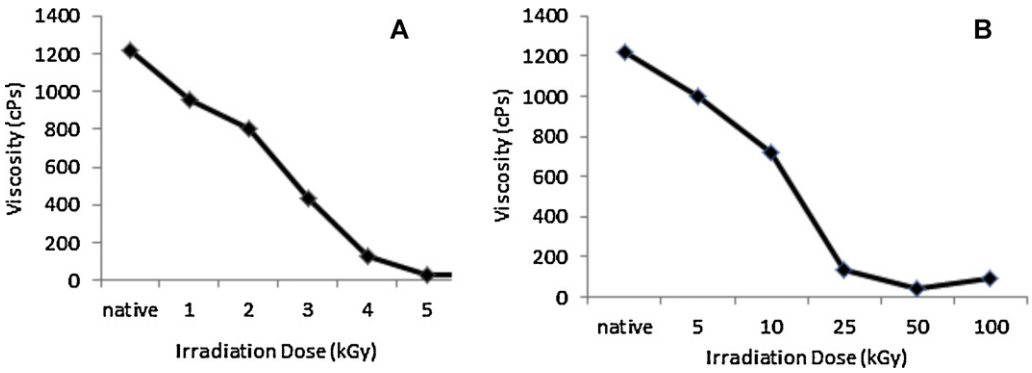

Fig. 1. Impact of gamma irradiation on viscosity of guar gum samples. (A) Irradiation of guar gum as aqueous solution (2% (w/v)); (B) irradiation of guar gum in powder form.

i.e. the time required for 50% reduction in mint oil content was calculated as $0.693/k$ (Durge et al., 2011).

2.2.9. Morphological characteristics by scanning electron microscopy (SEM)

The external structure of the encapsulated powder was studied by SEM (JSM 5800, JEOL, Tokyo, Japan). The powders were placed on the SEM stubs using a two-sided adhesive tape (Nisshin EM, Tokyo, Japan) and then analyzed after Pt–Pd sputtering by MSP-1S magnetron sputter coater (Vacuum Device, Tokyo, Japan). The dimensions of the particles were measured with help of an inbuilt scale present in SEM.

2.2.10. Statistical analysis

IBM® SPSS® statistic package was used for analysis of data. Analysis of variance (ANOVA) by Fisher's least significant difference was performed to examine the effect of irradiation on emulsifying and microencapsulation properties of gums. Principal component analysis was performed on relative areas of volatile compounds of mint oil as analyzed by gas chromatography using XLSTAT 2011 software. Since all the variables were on a same scale, covariance PCA was carried out. Loading and score plots were drawn using principal components 1 and 2.

3. Results and discussion

3.1. Effect of irradiation on molecular weight, polydispersity index and viscosity of guar gum

A dose dependent decrease in the viscosity of guar gum was observed on radiation (Fig. 1). Control guar gum had a viscosity of 1221 ± 4.72 cPs while IRS (5 kGy) and IRP (50 kGy) showed a viscosity of 30 ± 3.04 and 45 ± 1.99 cPs, respectively. An increase in the radiation dose beyond 5 kGy for IRS and 50 kGy for IRP did not decrease the viscosity any further. The lowering of viscosity in irradiated gum is attributed to irradiation induced depolymerization. These results are in good agreement with previous report of Jumel et al. (1996). Further, guar gum samples were analyzed by GPC and weight average molecular weight (M_w) and polydispersity index (PDI) were calculated. For both IRS and IRP, weight average molecular weight (M_w) decreased in a dose dependent manner (Fig. 2A and B). Polydispersity index (PDI) increased with an increase in irradiation dose (Fig. 2C and D). A maximum polydispersity index of 2.92 and 1.75 was observed for IRS (5 kGy) and IRP (50 kGy), respectively. Variation of weight average molecular weight (M_w) and relative area of all three peaks with radiation dose is depicted in Fig. 2E and F. Lower molecular weight peak (Peak 3) increased in a dose dependent manner in both IRS and IRP, while higher M_w peaks decreased in a dose dependent manner. The proportion of depolymerized fraction (Peak 3) was significantly ($p < 0.05$) higher in IRS than in IRP (Fig. 2E and F).

Fig. 3 depicts representative GPC chromatograms of native, PHGG, and irradiated guar gum both in solution (IRS 5kGy) and powder (IRP 50 kGy) form. Native guar gum and PHGG exhibited a single peak with weight average molecular weight (M_w) of 3.4×10^6 Da and 1.2×10^4 Da, respectively, while gamma irradiated samples were characterized by presence of three peaks in gel permeation chromatography profile. Peaks 1 and 2 were for native guar gum, while peak 3 was gamma depolymerized fraction of guar gum. Both viscosity and M_w data demonstrated the rate of degradation of guar gum to be greater when it was irradiated in solution form. Similar results were previously reported by Gupta et al. (2009). In solutions, the hydroxyl radical formed due to radiolysis of water abstracts the hydrogen from carbohydrate forming an unstable free radical which has very low probability of encountering another radical in dilute solution and hence it degrades into smaller fractions. On the other hand, during irradiation in powder form, guar gum degrades mainly due to direct effects resulting in lower rate of degradation (Gupta et al., 2009). Irradiation beyond 50 kGy in IRP and 5 kGy in IRS did not reduce the viscosity (Fig. 1) and molecular weight significantly (Fig. 2A and B). In fact, an increase in molecular weight (Fig. 2B) and viscosity (Fig. 1B) was observed in IRP at 100 kGy. This phenomenon may be due to radical-radical crosslinking in the guar gum as has been explained for gum Arabic (Al-Assaf, Sakata, McKenna, Aoki, & Phillips, 2009). Since the wall material for microencapsulation requires low viscosity at high concentration, further work was carried out with IRS at 5 kGy and IRP at 50 kGy.

3.2. Mint oil emulsion stability in gum Arabic alone and its blends with depolymerized guar gum as wall materials

The emulsification efficiency depends mainly on the emulsifying properties of the matrix and its ability to form films at the interfaces between the emulsion phases. Gum Arabic alone and combination of gum Arabic with IRS (5 kGy), IRP (50 kGy) at 90:10 and 80:20 and PHGG at 90:10 proportion showed good emulsification property with ESI value of 1 (Table 1) after 24 h of emulsion preparation. Since emulsion stability of all the wall materials could not be distinguished on the basis of ESI, turbidity of the emulsions was monitored. The turbidity of the emulsions prepared by a blend of gum Arabic with IRP or IRS was higher than that of gum Arabic alone indicating better stability of the emulsions prepared from blended wall materials (Table 1). However, blend of gum Arabic with PHGG showed lower turbidity of 0.41 than gum Arabic alone thus indicating its lower emulsion stability. This may probably be due to lower viscosity of the gum Arabic and PHGG blend. PHGG itself has poor emulsion stability, and blending it with gum Arabic

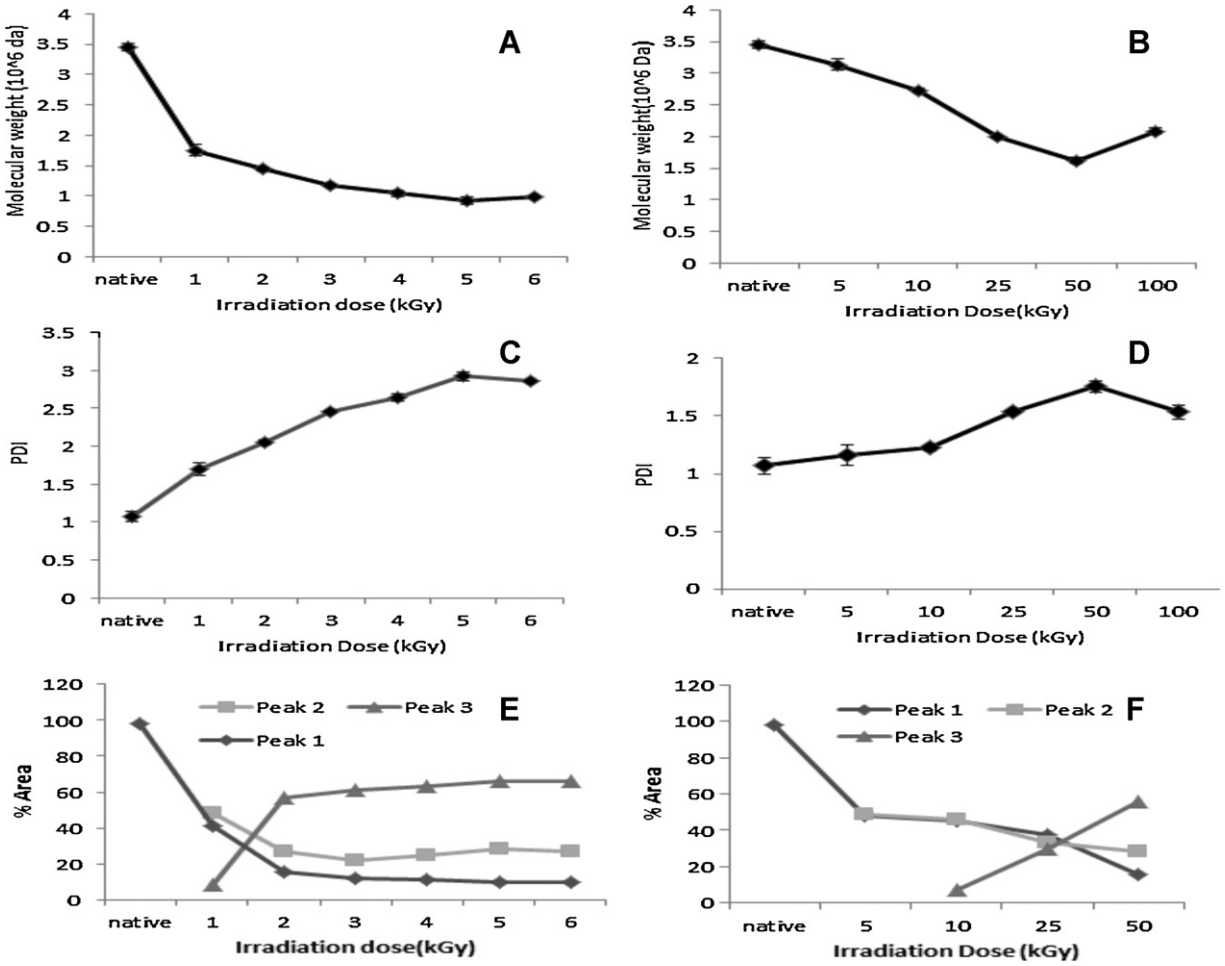

Fig. 2. Gel permeation chromatograms of guar gum. (A) Native guar gum; (B) guar gum irradiated in solution form (5 kGy); (C) guar gum irradiated in powder form (50 kGy); (D) enzymatically depolymerized guar gum (PHGG). Detector used: refractive index; injection volume 20 μL; column bio-basic SEC-1000; eluent deionized water; flow rate 0.6 mL/min.

decreased the emulsion stability of gum Arabic too. However, the blend of gum Arabic with both IRP and IRS had higher viscosity than gum Arabic alone, which may have stabilized the emulsion.

Particle sizes of emulsions prepared are given in Table 1. Gum Arabic blends with IRS or IRP showed a similar behaviour as gum Arabic alone (Table 1). Particle size of emulsions prepared was in the range of 12–14 μm. Furthermore, no increase in particle size was observed during storage. However there was marginal increase in particle size in emulsion prepared with PHGG as component from 12.89 ± 0.87 to 15.67 ± 0.67 μm after 24 h. Emulsions prepared with PHGG as wall component demonstrated lower emulsion stability as compared to gum Arabic alone or its blends with gamma depolymerized guar gum as wall materials.

The viscosity of the emulsion increased with an increase in the proportion of IRS and IRP in gum Arabic (Table 1). Gum Arabic blends (80:20) with IRS and IRP had higher viscosity of 141 ± 0.57 and 158 ± 2.60 cPs, respectively as compared to 33 ± 0.27 and 34 ± 0.33 cPs for gum Arabic alone and gum Arabic blend (90:10) with PHGG. The high viscosity of the emulsions made it difficult to spray and transportation by peristaltic pump of the spray dryer used in present study. Gum Arabic blend (90:10) with IRS and IRP had a viscosity of 97 ± 1.00 and 101 ± 0.95 cPs, respectively,

Table 1
Particle size,[a] ESI,[a] turbidity,[a] viscosity,[a] entrapment[a] and encapsulation efficiency[a] of emulsions prepared using different blends of carrier materials.

Wall materials	ESI	Turbidity	Emulsion particle size (μm)		Viscosity (cPs)	Encapsulation efficiency (%)	Entrapment efficiency (%)
			0 h	24 h			
Gum Arabic	1	0.56 ± 0.10^{b}	$12.76 \pm .29^{a}$	13.56 ± 0.94^{a}	33 ± 0.27^{a}	80.93 ± 1.44^{a}	85.44 ± 2.10^{a}
Gum Arabic:IRS (80:20)	1	0.87 ± 0.06^{c}	13.31 ± 0.49^{a}	13.78 ± 0.85^{a}	141 ± 0.57^{b}	ND	ND
Gum Arabic:IRS (90:10)	1	0.74 ± 0.11^{d}	12.95 ± 0.75^{a}	13.27 ± 0.33^{a}	97 ± 1.00^{c}	881.27 ± 1.29^{ab}	87.85 ± 1.78^{ab}
Gum Arabic:IRP (80:20)	1	0.95 ± 0.07^{e}	13.45 ± 0.86^{a}	13.57 ± 0.90^{a}	158 ± 2.60^{d}	ND	ND
Gum Arabic:IRP (90:10)	1	0.89 ± 0.12^{c}	12.22 ± 0.67^{a}	13.01 ± 0.46^{a}	101 ± 0.95^{e}	82.42 ± 2.17^{b}	88.12 ± 1.57^{b}
Gum Arabic:PHGG (90:10)	1	0.41 ± 0.17^{a}	12.89 ± 0.87^{a}	15.67 ± 0.6^{b}	34 ± 0.33^{a}	67.56 ± 2.62^{c}	70.13 ± 1.88^{c}

ND: not determined, since the emulsion was not spray dried.
Means in same columns with same superscripts do not differ significantly ($p < 0.05$).
[a] All values are mean ± SD of three or more determinations.

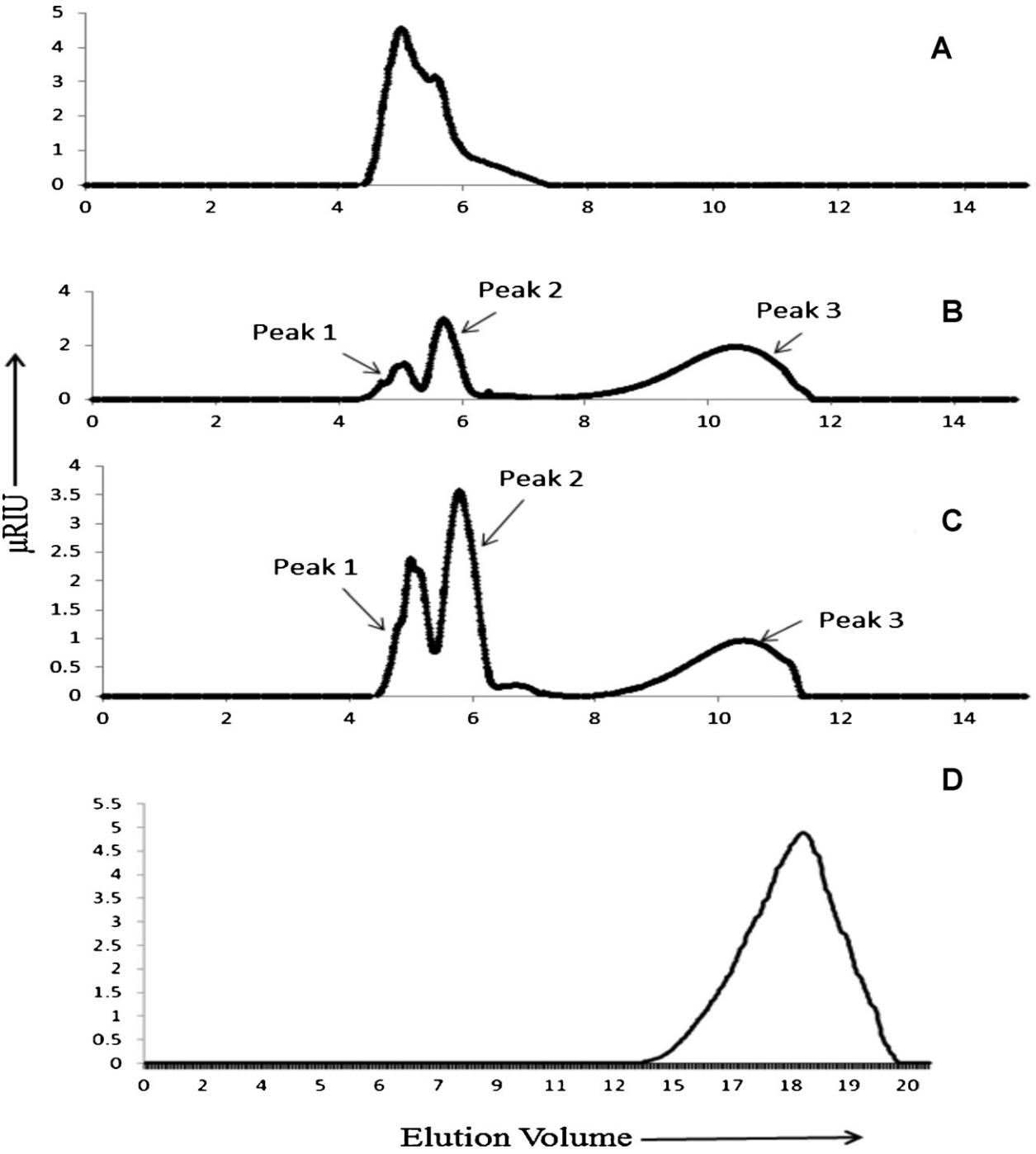

Fig. 3. Impact of gamma irradiation on weight average molecular weight (M_w) and polydispersity index (PDI) of guar gum samples. (A) Variation of weight average molecular weight (M_w) with gamma irradiation for guar gum irradiated as aqueous solution (2% (w/v)); (B) variation of weight average molecualr weight (M_w) with gamma irradiation for guar gum irradiated as powder; (C) variation of polydispersity index (PDI) with gamma irradiation for guar gum irradiated in solution form (2% (w/v)); (D) variation of polydispersity index (PDI) with gamma irradiation for guar gum irradiated in powder form; (E) variation of relative area percent of all three peaks observed in gel permeation chromatography profile with dose for guar gum irradiated in solution form; (F) variation of relative area percent of all three peaks observed in gel permeation chromatography profile with dose for guar gum irradiated in powder form.

and were found to be amenable to spray drying. Hence, only 10% replacement of gum Arabic was possible with IRS and IRP for microencapsulation of mint oil by spray drying. However, greater substitution of gum Arabic with gamma depolymerized guar gum might be possible in bigger industrial spray driers. Industrial spray driers can operate at higher pressure of the peristaltic pump and/or different nozzles (size as well the type) which was not possible in the laboratory spray drier that we used in our study.

3.3. Analysis of mint oil microencapsulated in gum Arabic and its blends (90:10) with IRS, IRP or PHGG

The microcapsules of mint oil obtained by spray drying in wall materials comprising of gum Arabic alone or its combinations (90:10) either with IRS, IRP or PHGG were analyzed for total oil and surface oil to calculate encapsulation efficiency and entrapment efficiency which are indicators of effectiveness of microencapsulation. Gum Arabic, gum Arabic:IRS (90:10), gum Arabic:IRP

Table 2
Regression analysis of free and encapsulated mint oil at 27 ± 2 °C.

Encapsulation material	Regression equation for storage stability	Half life $t_{1/2}$, 27 ± 2 °C (weeks)
Gum Arabic	ln (% retention of mint oil) = −0.027 time + 4.63 (R^2 = 0.923)	25.66
Gum Arabic:IRS (90:10)	ln (% retention of mint oil) = −0.020 time + 4.60 (R^2 = 0.982)	34.65
Gum Arabic:IRP (90:10)	ln (% retention of mint oil) = −0.018 time + 4.63 (R^2 = 0.939)	38.50
Gum Arabic:PHGG (90:10)	ln (% retention of mint oil) = −0.036 time + 4.11 (R^2 = 0.973)	17.11

(90:10) and gum Arabic:PHGG (90:10) showed encapsulation efficiency of mint oil of 80.93 ± 1.44, 81.27 ± 1.29, 82.42 ± 2.17 and 67.56 ± 2.62, respectively. Soottitantawat et al. (2005) reported similar retention of L-menthol (which is the main component in mint oil) in gum Arabic after spray drying. Surface oil is an important factor influencing the storage stability of the microcapsules as the surface oil can easily oxidize and result in unacceptable off-flavours. In the present study, gum Arabic gum, Arabic:IRS (90:10), gum Arabic:IRP (90:10) and gum Arabic:PHGG (90:10) showed entrapment efficiency of 85.44 ± 2.10, 87.85 ± 1.78 and 88.12 ± 1.57 and 70.13 ± 1.88, respectively (Table 1). There was no statistically significant ($p < 0.05$) difference between effectiveness of microencapsulation using gum Arabic alone or its blends with gamma depolymerized guar gum (IRS and IRP) as wall materials. Expectedly, gum Arabic blends with enzyme depolymerized guar gum (PHGG) demonstrated significantly lower ($p < 0.05$) encapsulation and entrapment efficiency as compared to all other wall materials.

Microcapsules were subjected to storage stability study for a period of 8 weeks at 27 ± 2 °C and analyzed for retention of total mint oil within the capsules. A linear nature of semi-log plot of percent retention of mint oil vs. storage time showed the loss of mint oil from the microcapsules to follow a first order kinetics (Fig. 4). The regression equation and $t_{1/2}$ values for gum Arabic and gum Arabic substituted partially with 10% IRS, IRP or PHGG is given in Table 2. Shaikh, Bhosale, and Singhal (2006) and Bule et al. (2010) reported first order kinetics for black pepper oleoresin and CoQ10 microcapsules during storage. The $t_{1/2}$ for mint oil in microcapsules of gum Arabic, gum Arabic:PHGG (90:10), gum Arabic:IRS (90:10), gum Arabic:IRP (90:10) were 25.66, 17.11, 34.65 and 38.50 weeks, respectively (Table 2). Microcapsules prepared using gum Arabic as sole wall material had a percent retention of 58.12 ± 1.56 after 8 weeks as opposed to 61.22 ± 1.59, 65.78 ± 2.22 and 48 ± 2.01 for gum Arabic:IRS (90:10), gum Arabic:IRP (90:10) and gum Arabic:PHGG (90:10), respectively.

Microcapsules having gamma depolymerized guar gum as wall material component demonstrated significantly ($p < 0.05$) higher $t_{1/2}$ values and corresponding higher retention of mint oil after 8 weeks of storage as compared to microcapsules having gum Arabic alone as wall materials. Gum Arabic:IRP (90:10) showed better retention of mint oil than gum Arabic:IRS (90:10). Interestingly, microcapsules formed with gum Arabic and PHGG demonstrated significantly lower ($p < 0.05$) $t_{1/2}$ and mint oil retention after 8 weeks of storage. Better retention of mint oil in microcapsules formed with gamma depolymerized guar gum as a wall material component might be due to the fact that irradiated samples had high M_w fractions (3.84×10^6 Da) apart from depolymerized fraction. High M_w fractions might have resulted in better film forming ability and consequently better retention of mint oil within the microcapsules. Better retention demonstrated by microcapsules with IRP as compared to IRS might be due to the fact that IRP had greater proportion of high M_w fractions as compared to IRS (Fig. 2E and F). Poor retention of mint oil in gum Arabic:PHGG (90:10) microcapsules might be due to presence of only lower molecular weight fraction of 1.2×10^4 Da in PHGG.

Principal component analysis (PCA) was carried out to identify qualitative changes in mint oil entrapped in Gum Arabic, gum Arabic:IRP (90:10) and in gum Arabic:IRS (90:10) during 56 days storage. First two principal components explained 77.63% and 14.20% of data variance (PC1 and PC2, respectively). Loading and score plots are depicted in Fig. 5. PC1 was highly influenced by menthol, α-pinene, β-pinene, limonene, 3-octanol while compounds contributing more towards PC2 were neo iso menthone, pulegone, iso menthone, menthone, 1,8 cineole and methyl acetate (Fig. 5A). The score distribution from first two PCs (Fig. 5B) demonstrated three separate groups in samples analyzed. First group had samples

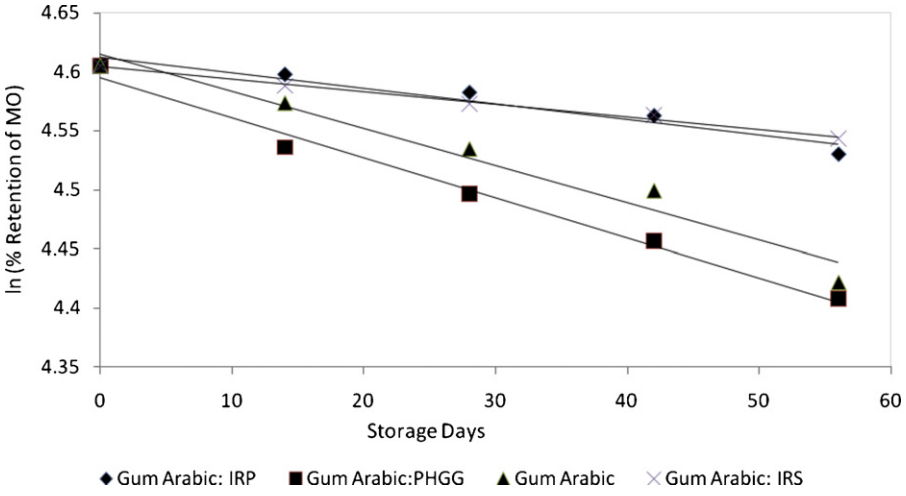

Fig. 4. Stability of encapsulated mint oil measured as ln retention of mint oil vs. storage time in days (A) Gum Arabic:IRP; (B) gum Arabic:PHGG; (C) gum Arabic; (D) gum Arabic:IRS; stored at 27 ± 2 °C.

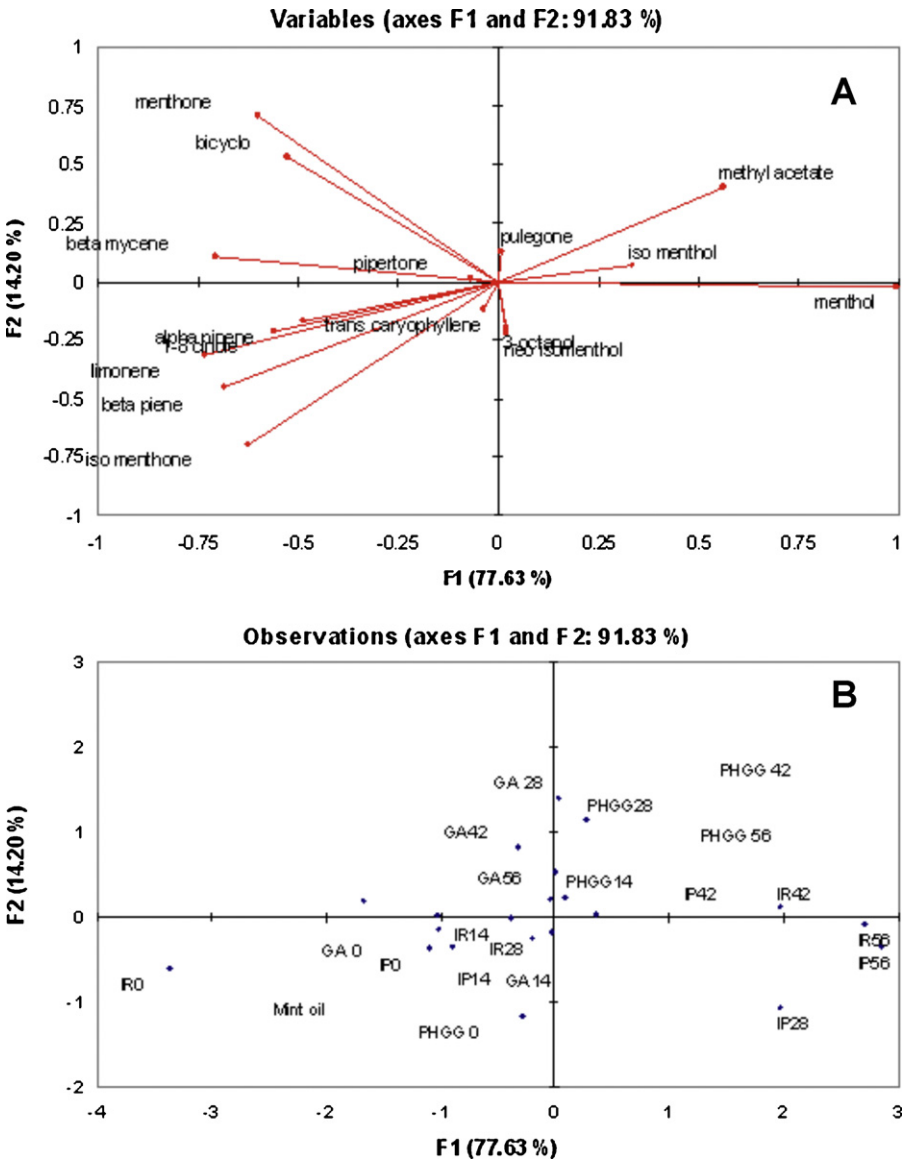

Fig. 5. Principal component analysis of volatiles of mint oil encapsulated with different wall materials after various storage periods (plots of first two principal components). (A) Loading plot depicting distribution of various mint oil compounds with F1 and F2; (B) score plot depicting distribution of various samples with F1 and F2.

(IRS0, IRP0, GA0, PHGG0, IRS14, IRP14, GA14, IRS28 and mint oil (MO)) in initial storage period (up to 14 days) and was located on negative side of both PC1 and PC2. GA28, GA42 and GA56 constituted second group located on negative side of PC1 but positive side on PC2 and third group (PHGG14, IRP28, PHGG28, IRP42, PHGG42, IRS42, PHGG56, IP56, and IR56) was located on positive side of both PC1. Interestingly, in the later stages of storage (beyond 14 days), samples having gum Arabic as wall material evolved separately from samples having depolymerized guar gum as wall material. From loading plot (Fig. 5A) it can be concluded that monoterpene hydrocarbons like α-pinene, β-pinene, limonene, 1,8 cineole and menthone are correlated with the first group while oxygenated monoterpenes like menthol and neo isomenthol are positively correlated to third group. It can be concluded that during storage there was a decrease in the content of monoterpene hydrocarbons with corresponding increase in oxygenated monoterpenes like menthol. Further, samples having guar gum as wall material component demonstrated better retention of menthol and isomenthol as compared to gum arabic alone as wall material. These results suggest partial replacement of gum Arabic by 10% IRS and IRP to be a practical proposition.

3.4. Morphological characterization by scanning electron microscopy (SEM)

SEM was used to investigate the morphological structure of microcapsules prepared from gum Arabic, gum Arabic:IRS (90:10) and gum Arabic:IRP (90:10) (Fig. 6). All the microcapsules showed both grooves and smooth surfaces. The particle size ranged from 2.12 to 15.13 μm for gum Arabic, 2.47–16.11 μm for gum Arabic:IRP (90:10), and 2.41–14.13 μm for gum Arabic:IRS (90:10). This again suggested no significant change in the particle dimensions or appearance of microcapsules of mint oil prepared from gum Arabic and that substituted at 10% with radiation depolymerized guar gum.

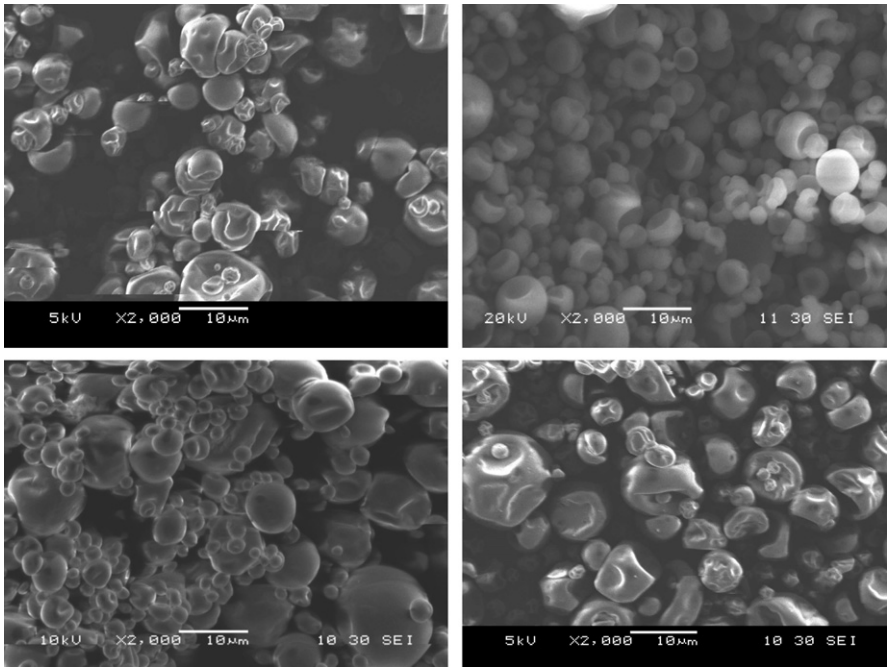

Fig. 6. Scanning electron microscopy of the microcapsules of mint oil obtained after spray drying with following wall materials (A) gum Arabic; (B) gum Arabic:IRS; (C) gum Arabic:IRP; (D) gum Arabic:PHGG, magnification 2000×.

4. Conclusion

Irradiation of guar gum decreased its viscosity and molecular weight but increased the polydispersity index. Substitution of gum Arabic with 10% radiation depolymerized guar gum showed no significant alterations in emulsion characteristics, but increased the stability of mint oil in the microcapsules as evident from the percent retention and half-life of mint oil within the microcapsules. However, decreased stability of mint oil in microcapsules prepared with PHGG as wall material component was observed. Irradiation of guar gum directly in the powder form was convenient and feasible as compared to irradiating it in solution form. Irradiation in solution form would also necessitate an additional drying step which is completely avoided on irradiation of guar gum in powder form. Hence, guar gum irradiated at 50 kGy could be used as partial replacement of gum Arabic for encapsulation of sensitive food ingredients.

Acknowledgements

Authors are grateful to University Grant Commission, Government of India, for providing financial assistance during the course of this investigation.

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