Available online at www.sciencedirect.com
ScienceDirect
Journal of Colloid and Interface Science 311 (2007) 253–261
JOURNAL OF Colloid and Interface Science
www.elsevier.com/locate/jcis

Effects of gamma radiation on phase behaviour and critical micelle concentration of Triton X-100 aqueous solutions

G. Valdés-Díaz [a], S. Rodríguez-Calvo [a], A. Pérez-Gramatges [a,*], M. Rapado-Paneque [b], F.A. Fernandez-Lima [c], C.R. Ponciano [d], E.F. da Silveira [d]

[a] *Department of Radiochemistry, Instituto Superior de Tecnología y Ciencias Aplicadas, Quinta de los Molinos, A.P. 6163, Ciudad de La Habana, Cuba*
[b] *Center of Technological Applications and Nuclear Development, 5ª y 30, Ciudad de La Habana, Cuba*
[c] *Division of Advanced Studies, Instituto Superior de Tecnología y Ciencias Aplicadas, Quinta de los Molinos, A.P. 6163, Ciudad de La Habana, Cuba*
[d] *Physics Department, Pontifícia Universidade Católica, Rio de Janeiro, Brasil*

Received 21 January 2007; accepted 25 February 2007

Available online 2 March 2007

Abstract

Ionising radiation used for sterilization can have an effect on the physicochemical properties of pharmaceutically relevant excipient systems, affecting therefore the stability of the formulation. The effect of gamma irradiation on the phase behaviour (cloud point—CP) and critical micelle concentration (CMC) of aqueous solutions of Triton X-100, used as a model nonionic surfactant, is investigated in this paper. Micellar solutions were irradiated with γ-rays in a dose range between 0 and 70 kGy, including the sterilization range of pharmaceutical preparations. The decreased observed in CP and CMC values of micellar solutions at all absorbed doses was explained in terms of changes in molecular mass distribution of ethoxylated surfactant and the formation of cross-linked species. These results were complemented by mass spectrometry, UV–vis and NMR spectroscopy. Although the findings indicate degradation of polyethoxylated chains by water radical attacks, there was no spectroscopic evidence of radiation damage to aromatic ring or hydrocarbon tail of surfactant. Models based on Flory–Huggins theory were employed to estimate CP from changes in mass distribution and to obtain cross-linking fractions. Surface tension measurements of non-irradiated and irradiated solutions were used for estimating the effectiveness and efficiency of surfactant in the formulation.

Keywords: Surfactant; Gamma irradiation; Cloud point; CMC; Mass spectrometry; Formulations

1. Introduction

Nonionic surfactants are commonly used as excipients in the pharmaceutical industry for a wide range of applications in formulations as wetting agents, emulsifiers, or solubilisers [1]. In protein formulations, surfactants minimize adsorption into surfaces and reduce the air–liquid interfacial surface tension and thus the rate of protein denaturation [2]. Surfactants are often utilized in formulations above their critical micelle concentration (CMC) values. For example, recombinant human factor XIII is protected against both agitation and freeze-thaw-induced aggregation in the presence of polysorbate 20 at concentrations corresponding to the CMC (0.007%, w/v) [3]; however, at concentrations below this CMC value, it is not stabilized. It has also been reported that surfactant degradation products can impact the stability of the formulation [2]. Therefore, a thorough characterization of the stability of all the formulation components is essential for a successful product.

Radiation sterilization of biopharmaceutical products is a common routine because the biological contaminants are totally inactivated by the exposure to high doses [4]. However, its use has been recently restricted to some applications, since the radiation sterilization causes either a slight variation in molecular weight or degradation of some major components, e.g. polymers, hydrogels, etc. [5]. When the sterilization process is performed in solutions where surfactants are used, similar results can be expected. In particular, research on the radiation effects in drug formulations and surfactants–polymers systems is necessary to estimate how the functions of surfactants as

* Corresponding author. Fax: +55 537 2041188.
E-mail address: apgram@instec.cu (A. Pérez-Gramatges).

0021-9797/$ – see front matter
doi:10.1016/j.jcis.2007.02.081

emulsifiers and solubilisers can be affected. In such systems, the interaction between surfactants and proteins is of central importance in the preparation of a broad range of pharmaceutical products, where high sterilization doses are routinely used [6,7].

Studies on radiation chemistry in micellar systems are mainly referred to their importance as pollutants in water ecosystems [8]. There are several reports on degradation processes of alkylphenyletoxylated surfactants in aqueous solutions via water radicals after gamma irradiation [9–12]. The results showed that the two surfactant domains (head and tail) are exposed to the attack of water radicals. In particular, literature reports that in nonionic surfactants of the polyoxyethylene–*t*-octylphenyl ether family (Triton), the aromatic ring in the hydrophobic tail and the ethoxylated (EO) groups in the hydrophilic head seem to be the most likely sites for radical attack [9,11]. In all cases, the hydroxyl radicals have been depicted as the principal species initiating and propagating reactions in AOPs [11], even when the presence of oxygen in non-de-aerated systems can increase the decomposition yield almost two times higher [12]. If the attack occurs to the ring, an adduct is formed and, after several attacks, the ring breaks. When the attack occurs to EO groups, shorter ethoxylated surfactant molecules are obtained. It has also been reported that at concentrations below the CMC, the attack to the ring is ten times faster than the attack to EO groups [9]. This fact usually does not occur above the CMC, due to the shielding effect produced by micellization; only changes in the EO groups are expected after moderate gamma irradiation [13].

Despite the attention devoted to the study of degradation by irradiation of surfactants of environmental interest, studies of the effect of gamma radiation on physicochemical properties of aqueous surfactant solutions are not widely reported. On the other hand, some attempts have been made to identify the physicochemical parameters of surfactants that would be predictive for their absorption-enhancing properties in formulations to increase the absorption of drugs by different organs [14]. These parameters included hydrophilic–lipophilic balance (HLB), CMC, surfactant structure, and the surfactant surface activity [15–17]. Other investigations of liquid formulations containing nonionic surfactant have showed that the cloud point of the surfactant can be a key parameter for consideration, and it can be modulated through the proper choice of excipient [18]. Phase separation of the surfactant can also be used beneficially in pharmaceutical formulations, such as in the case of gelation of poloxamer solutions and its potential applications in the controlled-release of drugs through various routes of delivery [19].

In the present work the effect of gamma-irradiation on the cloud point and the critical micelle concentration of micellar aqueous solutions of Triton X-100, used as a model surfactant solution, was studied. Triton X-100 (*a.k.a.* octoxynol 9) is a commercially available surfactant used as a solvent detergent in numerous pharmaceutical applications including virus inactivation [20]. The cloud point (CP), a characteristic temperature in which micellar systems of nonionic surfactants show a reversible separation phenomenon, is a unique property that

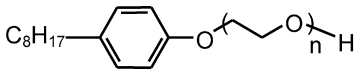

Fig. 1. Chemical structure of Triton X-100 ($n = 9.5$).

characterizes the fine balance between hydrophilic (head) and hydrophobic (tail) interactions occurring in a micellar solution [21]. It is thus highly dependent on surfactant chemical structure, since in the case of surfactants containing EO units, the bigger the size of head, the higher the CP; the opposite applies to the hydrophobic tail. The CMC, another important characteristic in surfactant systems, is a sharply defined concentration region where micelle formation occurs [21]. Similarly to the CP value, the structure of the surfactant has also a substantial influence on CMC. The surface tension behaviour of surfactant solutions can also be employed to obtain other relevant parameters that characterize the performance in a formulation, such as effectiveness and efficiency of the surfactant.

Irradiation was performed in a wide range of absorbed dose, including those commonly used for sterilization. Complementary techniques, namely mass spectrometry, NMR, IR, and UV spectroscopy, were also used in this work to assess the effect of radiation on the surfactant structure, in order to evaluate and understand the possible changes in the physicochemical properties and their effect on the surfactant performance in a formulation.

2. Materials and methods

2.1. Reagents

The nonionic surfactant used was polyoxyethylene–*t*-octylphenyl ether, with an average of 9.5 oxyethylene (EO) units per molecule (Triton X-100, Fig. 1), from Rohm & Haas Co. Surfactant concentration was 1 wt%, ca. 1.6×10^{-2} mol/dm^3, which is well above the CMC of Triton X-100 (2.4×10^{-4} mol/dm^3, [22]); the percentage of surfactant is based on the amount of water present (in all the experiments distilled water was used). The surfactant was used as received. The mixtures were prepared 24 h in advance to ensure full hydration of micelles. The cloud point under these conditions was 64.5 °C.

2.2. Gamma irradiation of the samples

Samples were irradiated in non-de-aerated glass ampoules using gamma rays from a ^{60}Co gamma source, at 25 °C. The activity of the radiation chamber was 2.12 kCi and the dose rate was 1.373 kGy/h, measured by Fricke and ceric sulfate dosimeters. Small aliquots of Triton X-100 aqueous solutions (1 wt%) were irradiated at doses between 0.1 and 70 kGy.

2.3. Cloud point determination

The temperature at which the cloud point phenomenon occurs was determined by the method reported by Carvalho and Briganti [23]. The method is based on the visual observation of the separation of phases in the micellar solution. The initial solution was heated in a water bath (MLW thermostat by VEB

Elektro bad Franken Hausen) at temperatures well above its cloud point (turbid solution). After that, the solution was gradually cooled with constant stirring and keeping a stable temperature. The cloud point (CP) was considered as the temperature at which the solution became clear. To verify the results, the opposite process was carried out by gradually heating the clear solution until turbidity appeared. The reported value was the average of four determinations. Precision of temperature measurements in terms of standard deviation was ±0.2 °C.

2.3.1. Cloud point theoretical analysis

Using the approximation described by Rupert [24] and Inoue et al. [25] (as an extension of the Flory–Hugging theory for polymer solutions), the CP can be expressed as:

$$\mathrm{CP} = \frac{\phi_{\mathrm{m}}^2 H_{12}}{R[\ln(1-\phi_{\mathrm{m}}) + (1-(1/N))\phi_{\mathrm{m}}] + S_{12}\phi_{\mathrm{m}}^2}, \tag{1}$$

where H_{12} and S_{12} are the enthalpy and the entropy of the monomer interaction with water, respectively; ϕ_{m} is the volume fraction of the surfactant; and N is the aggregation number of the micelle.

In order to apply this equation to our system, four assumptions were initially made: (i) the number of cross-linked species, and therefore its effect on the CP, is negligible; (ii) there is not variation in the surfactant tail structure due to radical attack; (iii) Triton X-100 behaves as monodisperse surfactants with an EO chain length equal to the mean value of the EO distribution at each dose; and (iv) the entropy of mixing remains constant with the absorbed dose. The former mathematical expression (Eq. (1)) can then be rearranged as:

$$\mathrm{CP} = \frac{H_{\mathrm{eff}}^{\mathrm{w}}}{\Delta S_{\mathrm{mix}} + S_{\mathrm{eff}}^{\mathrm{w}}}, \tag{2}$$

where

$$\Delta S_{\mathrm{mix}} = \frac{R[\ln(1-\phi_{\mathrm{m}}) + (1-1/N)\phi_{\mathrm{m}}]}{\phi_{\mathrm{m}}^2}$$

is the entropy of mixing of the system and $H_{\mathrm{eff}}^{\mathrm{w}}$ and $S_{\mathrm{eff}}^{\mathrm{w}}$ are H_{12} and S_{12}, respectively.

Inoue et al. [25] also used the Flory–Huggins theory in order to obtain a theoretical equation for the cloud point that takes into account the presence of an additional surfactant and forming mixed micelles:

$$\mathrm{CP} = \frac{[H_{12} + (H_{13}-H_{12})x_3 - (1-x_3)x_3 w_{23}]\phi_{\mathrm{m}}^2}{R[\ln(1-\phi_{\mathrm{m}}) + (1-(1/N))\phi_{\mathrm{m}}] + [S_{12} + (S_{13}-S_{12})x_3]\phi_{\mathrm{m}}^2}. \tag{3}$$

In this case, H_{12}, H_{13}, S_{12}, S_{13} are the interaction parameters of both surfactants with water in non-mixed systems; ϕ_{m} is the volume fraction of the surfactants; N is the aggregation number of the mixed micelles; w_{23} is the free energy of interaction between both surfactants; and x_3 is the mole fraction of the surfactant added, on surfactant only basis.

2.4. CMC and superficial tension measurements

The critical micelle concentration (CMC) was obtained using surface tension (γ) measurements. Progressive aliquots of surfactant stock solution (1 wt%, irradiated or not) were added to a volume of 50 mL of water in a thermostatic cell using a micropipette. Surface tension was measured after each addition using a Nima Technology Ltd tensiometer which works using the Wilhelmy plate method. The instrumental error was ±1 mN/m. The CMC was obtained as the intersection of the two linear fits relative to the initial surface tension depression and the further plateau, as reported elsewhere [26]. Surface tension measurements were also used to obtain the effectiveness (Γ_{m}) and the efficiency ($\mathrm{p}C_{20} = -\log C_{(-\Delta\gamma=20)}$) of the surfactants, following the method reported in Ref. [27].

2.5. Mass spectrometry analysis

Commercial polyethoxylated surfactants, such as Triton X-100, are obtained as a polymeric distribution having the same tail structure but different head, with a mean value of EO groups of 9.5 [28]. The characterization of polymers by mass spectrometry using laser vaporization/ionization sources has shown that the molecular mass distribution (MMD) is sensitive to instrumental and sample preparation parameters used to obtain the polymer mass spectrum [29]. It has been shown that the MMD depends on the matrix and on the laser energy [30].

The mass spectrometry analysis was performed in the reflective mode of a BRUKER/BIFLEX III mass spectrometer, equipped with a 337 nm UV nitrogen laser (3 ns FWHM, 200 μJ mean energy per pulse) from Laser Science Inc. Samples were analyzed using laser desorption ionization (LDI) and matrix assisted laser desorption ionization (MALDI). The standard dried droplet method was used for the sample preparation and a TX-100 typical concentration of 10^{-4} w/w was used. The TX-100 molecule ionization was ensured by applying small quantities of Na and K ions in the sample solution. For the LDI and MALDI analyses a laser intensity of 0.75 and 0.15 GW/cm^2 was used, respectively. The 4-hydoxy-α-cyanocinnamic acid (α-CHCA) was used as a matrix for the MALDI analysis, with a concentration of analyte (TX-100) to matrix (α-CHCA) molecules of 1:10. All the mass spectra correspond to an average of 15 and 10 laser shots for LDI and MALDI analyses, respectively.

2.6. UV–vis and NMR spectroscopy analysis

UV–vis spectra of non-irradiated and irradiated solutions (35 and 70 kGy) were obtained using a Varian Cary 50 probe spectrometer and quartz cuvettes. The analytical peak was observed at the characteristic wavelength of 275 nm, indicative of the presence of aromatic moiety in the surfactant structure. ^{13}C NMR spectroscopy analysis (PENDANT spectra) was performed in an AV500 BRUKER spectrometer.

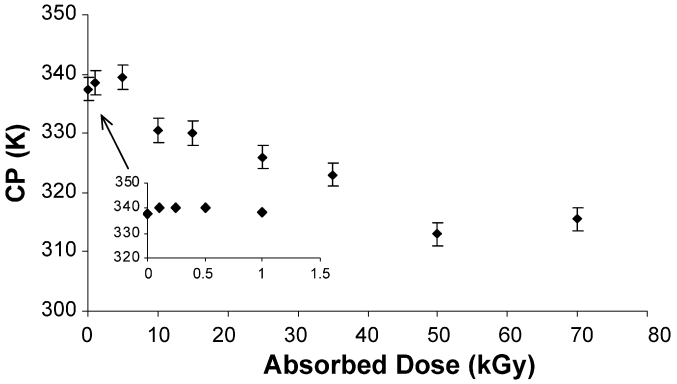

Fig. 2. Effect of gamma radiation on the phase behaviour of micellar solutions of Triton X-100 (1 wt%).

3. Results and discussion

Irradiation using gamma rays can induce significant changes in the macroscopic properties of micellar solution of Triton X-100, and thus affect the scope of their applications. In order to study this effect, three physicochemical properties were evaluated as a function of the absorbed dose: cloud point (CP), CMC and surface tension. These macroscopic quantities are of great use to describe the surfactant behaviour in cosmetic and pharmaceutical formulations, as well as the suitability of different surfactants to prevent protein surface adsorption in several applications.

3.1. Effect of gamma radiation on the cloud point

Two main regions can be depicted in the phase behaviour after irradiation (Fig. 2): a first region (0.1–5 kGy), where the CP value does not show significant changes, and a second region (5–50 kGy), characterized by a roughly linear decrease of CP. It is worthy to note that at higher doses (70 kGy), the CP value remains almost constant, and that the range where greater changes are observed is precisely the one comprising typical sterilization doses (15–30 kGy).

Cloud point variations as a function of the irradiation dose are closely related to changes in the surfactant structure, probably as consequence of the indirect action of gamma radiation on surfactant molecules, i.e. the interaction with free radical products of the water radiolysis. The direct interaction is less efficient, considering that the irradiation was performed in a dilute aqueous solution.

For the indirect interaction, there are three possible domains in the chemical structure of Triton X-100 (Fig. 1) that can be sensitive to these radical attacks: the hydrocarbon chain, the aromatic ring and the EO polymeric unit. However, considering that these solutions were well above CMC of surfactant, it is logical to assume that most of the primary degradation of the surfactant molecule would occur on the polyoxyethylene chain (EO), due to the shielding effect that the ethoxylated groups have over the tail [13]. A similar decrease was reported by Pelizzetti et al. for the mean value of the EO groups, due to the action of hydroxyl (OH) radicals in an aqueous system of a non-ionic ethoxylated surfactant (Igepal CO-720, 6.0×10^{-4} mol/L

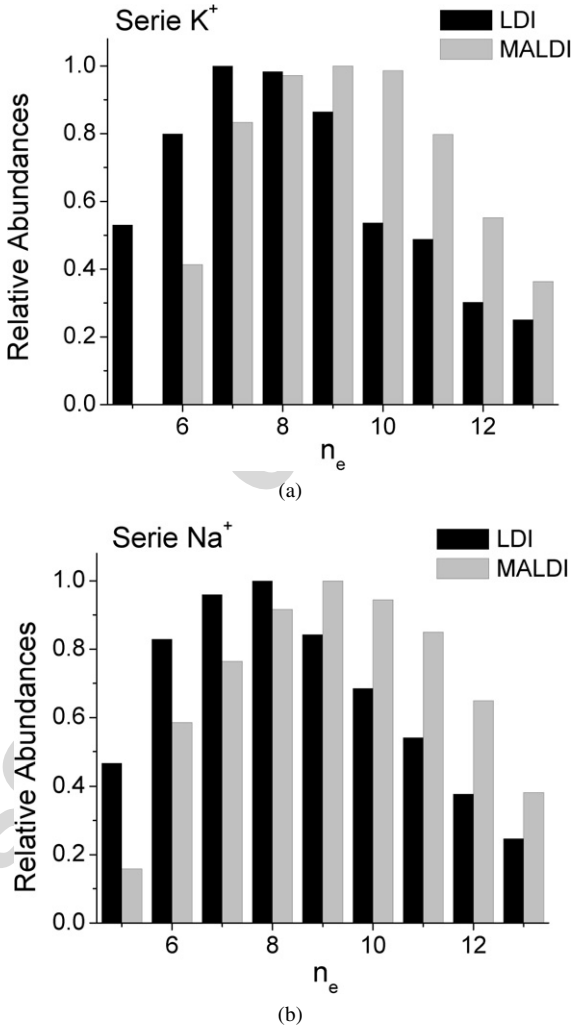

Fig. 3. LDI and MALDI molecular mass distribution: (a) MnK^+ and (b) $MnNa^+$ of non-irradiated Triton X-100.

[9]). In an early report on effects of gamma irradiation upon aqueous solutions of different kinds of surfactants, it was found that bond cleavage of oxyethylene in polyoxyethylene surfactant (POE) was the main chemical reaction occurring after radiolysis of water [11].

To investigate the effect of the irradiation dose in the structure of surfactants, a mass spectrometry analysis of this polymeric distribution was performed, using LDI and MALDI techniques (Fig. 3). A shifted MMD distribution to lower EO groups is obtained when using LDI. Moreover, the MALDI analysis shows that the mean value of the EO groups is of the order of 9–10, as expected from the commercial data. In this sense, the structural changes induce by the irradiation in the MMD distribution of the TX-100 can be probed by using MALDI technique ("soft ionization"). A more adequate analysis of the MMD distribution using the peak area as a function of the number of EO groups (n_e) gives an average number of 9.3 (Fig. 4).

The MMD mean values of the irradiated samples were calculated using the peak areas (Fig. 5). The n_e values for very low doses (less than 1 kGy) show an average value of 9.25 ± 0.31, and a slight decrease is observed towards a dose value of

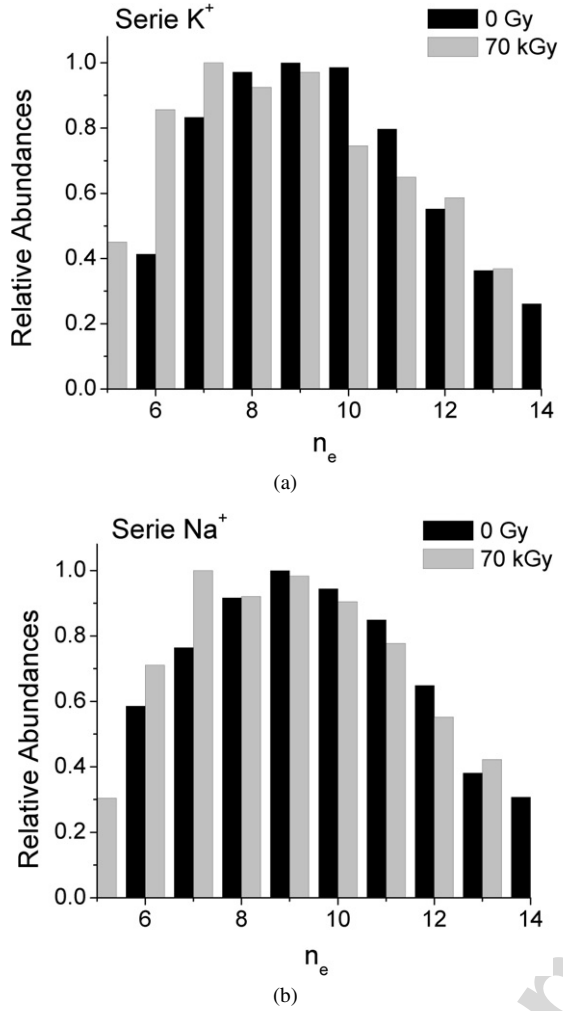

Fig. 4. Molecular mass distribution (MMD) of EO groups (n_e) for the (a) non-irradiated and (b) 70 kGy irradiated Triton X-100 samples. The data were acquired from the MALDI mass spectra.

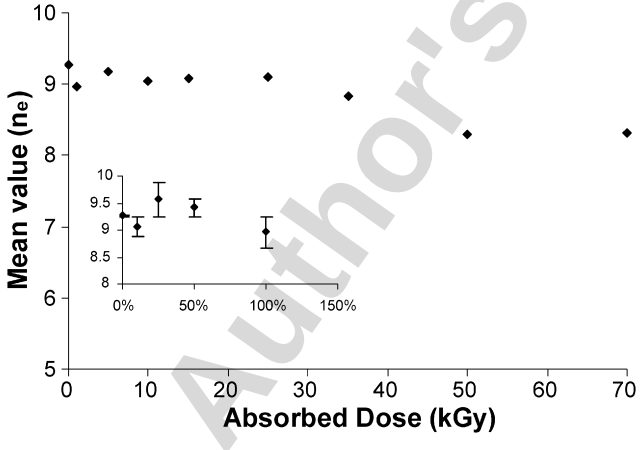

Fig. 5. Mean distribution values of EO groups of Triton X-100 micellar solutions (1 wt%) as a function of the absorbed dose.

50 kGy. For larger doses, the change in the MMD mean values relative to the non-irradiated sample is equivalent to losing one group in the EO polymeric chain.

In the case of the surfactant hydrocarbon tail, there are no evidences of fragmentation at these absorbed doses (up to 70 kGy). Other complementary results were obtained using NMR and UV–vis spectroscopy (see supplementary information). The results from ^{13}C NMR analysis did not show any significant differences among the spectra of non-irradiated and irradiated samples. In the case of UV–vis results, two characteristic peaks were observed at 225 and 275 nm, which can be related to the aromatic moiety in the hydrocarbon tail of the surfactant [11]. The three spectra showed the same absorbance maxima at the characteristic wavelengths, indicating that there were not significant changes in the structure of the substituted aromatic ring. The intensity of the peak at 275 nm remained invariable as the dose increased, in comparison to the non-irradiated samples. This peak was used for comparison purposes to determine if there was fragmentation of the ring due to radical attack. The higher intensity peak observed at 225 nm shows a significant interference from other peaks in the near UV region, where other aromatic transitions take place, and water and quartz absorb, thus quantitative measurements are not reliable [31]. For the surfactant concentrations used in this study (above CMC), the arrangement of EO head groups at the micellar surface behaves as a mechanic-structural barrier that limits the exchange of species between the micellar interior and bulk water. In this case, the occurrence of typical radical reactions on the micellar core is restricted, and the chemical structure of the hydrocarbon core does not experience significant changes [13].

Since there were not evident changes in the tail of the surfactants after irradiation in the absorbed dose range studied, and taking into account the results from spectroscopic measurements, the decrease in the CP in the second region can be attributed to structural changes in the hydrophilic head. These structural changes can be related, at some extent, to a decrease in the mean value of the EO group distribution.

Considering the assumptions mentioned in Section 2.3.1, H^w and S^w are the most significant parameters in Eq. (1). These thermodynamic quantities can be understood as effective parameters that can differ from those obtained for Triton X-100 non-irradiated solutions:

$$H^w_{eff} = H^w_0 + \Delta H(D_{abs}), \tag{4}$$

$$S^w_{eff} = S^w_0 + \Delta S(D_{abs}), \tag{5}$$

where H^w_0 and S^w_0 are the interaction parameters of Triton X-100 without irradiation, H^w_{eff} and S^w_{eff} are the interaction parameters of the surfactant after receiving a dose D_{abs}, and $\Delta H(D_{abs})$ and $\Delta S(D_{abs})$ are their variation with the absorbed dose.

A fitting procedure similar to that described by Rupert [24] was used to obtain H^w_0 and S^w_0 from the Triton X-100 miscibility curve (Fig. 6). The small divergence found at high volume fractions can be understood considering the deviation from ideal behaviour of the mixing entropy at higher concentrations. In those cases, a greater number of micelles have to be concentrated in a small volume during the phase separation. The values obtained for H^w_0 and S^w_0 are 3550 cal/mol and 10.95 cal/mol, respectively.

In order to estimate the value of $\Delta H(D_{abs})$ and $\Delta S(D_{abs})$, an approximation that accounts for the effect of different struc-

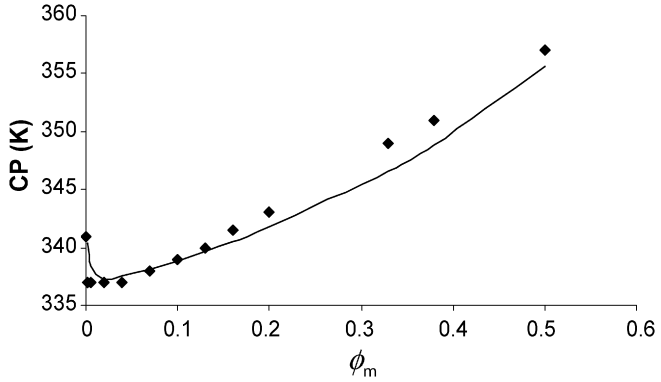

Fig. 6. Miscibility curve of Triton X-100 calculated according to Eq. (1). Experimental points were taken from Ref. [32].

Table 1
Comparison of experimental and calculated cloud point values (CP) of Triton X-100 solutions (1 wt%), using estimated interaction parameters

	Dose (kGy)								
	0	1	5	10	15	25	35	50	70
CP_{exp} (±2 K)	337.5	338.5	339.5	330.5	330	326	322	313	314.5
CP_{calc} (K)	337	334	337	335	335	335	333	327	327

tural groups on the interaction parameters, reported by Inoue et al. was used. This approximation describes H^w and S^w as functions of the numbers of EO (n_e) and carbon groups (n_c) in polyethyleneglycol (PEG) type surfactants [25]. Since no significant changes have occurred in the tail with the absorbed dose, the influence of the hydrocarbon tail on the interaction parameters remains constant. Therefore, the variation of H^w_{eff} and S^w_{eff} with the dose can be described as:

$$H^w_{eff} = H^w_0 + 610.58\Delta n_e, \quad (6)$$

$$S^w_{eff} = S^w_0 + 1.57\Delta n_e, \quad (7)$$

where Δn_e is the variation of the number of EO groups with the absorbed dose respect to the mean value in Triton X-100 without irradiation. Then, using the results from these calculations and the mass spectrometry results, the CP values were calculated (see Table 1).

A good agreement between the estimated and measured CP values is observed up to a dose of 15 kGy. Calculations for higher doses give significant differences, being greater than 10 K at the highest values. Under these conditions, it seems that the simple description of the water radical attack to surfactant molecules assumed up to this point can not be the only factor invoked to explain the radiation effect in the CP behaviour, in particular for absorbed doses above 15 kGy.

3.1.1. Influence of cross-linking reactions on cloud point

The approximation used to calculate the effect of gamma radiation on cloud point (Eq. (1)) includes the consideration that the number of cross-linked species was negligible. However, at higher doses, this contribution eventually becomes more important, since the probability of this type of reactions increases [13].

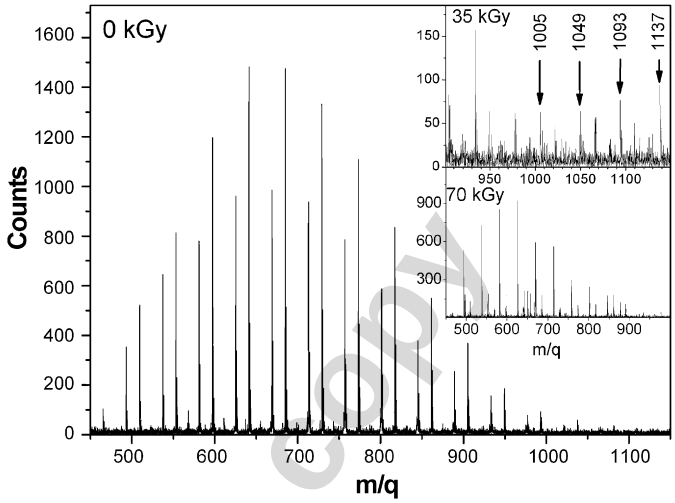

Fig. 7. MALDI spectra of non-irradiated and selected irradiated surfactant solutions (insets, 35 and 70 kGy). Peaks of possible cross-linking products are pointed in 35-kGy spectrum.

Other significant peaks in the mass spectra were observed at m/z ratios corresponding to various possible structures resulting from cross-linking reactions between surfactants radicals formed during the irradiation process (Fig. 7). The most likely structures for these cross-linked species are those resulting from surfactant radical attacks to the terminal carbon of the hydrophilic chain, which agrees with the fact that there were not significant changes in UV and NMR spectra of irradiated samples compared to non-irradiated Triton X-100 (see supplementary information). The presence of turbidity in those samples irradiated at doses higher than 15 kGy corroborates the formation of these cross-linked species, a phenomenon already reported in the literature [11,13].

The changes detected in Triton X-100 chemical structure after gamma irradiation can thus be considered as a consequence of water radical attacks producing variations in the mean distribution value of the EO groups, and of cross-linking reactions between surfactants radicals.

The analysis of phase behaviour should consider that, due to the occurrence of cross-linking reactions, the micellar solution behaves as a mixed surfactant system containing two kinds of surfactant: a majority of Triton X-100 molecules, and a smaller but significant amount of cross-linked surfactants. Furthermore, these compounds exist in solution as a distribution of different monomers.

Considering that the interaction between both surfactants can be neglected with respect to the water–surfactant interaction, Eq. (3) can be rearranged into:

$$CP = \frac{H^w_{eff} + (H_{cross} - H^w_{eff})x_3}{\Delta S_{mix} + S^w_{eff} + (S_{cross} - S^w_{eff})x_3}, \quad (8)$$

where H_{cross} is the interaction parameter of the cross-linked surfactant surfactants with water (H_{13}) and x_3 is the mole fraction of the cross-linking fraction.

The variation of cross-linking fraction (x_3) with the dose can be then estimated, using the experimental values obtained for CP and the interaction parameters of the cross-linked surfactant,

Table 2
Fraction of cross-linking obtained using CP and CMC measurements, at different absorbed doses

	Dose (kGy)								
	0	1	5	10	15	25	35	50	70
CP-calculated fraction of cross-linking (x_3)	≈0	≈0	≈0	0.11	0.14	0.27	0.31	0.39	0.37
CMC-calculated fraction of cross-linking (x_3)	≈0	≈0	≈0	0.10	0.14	0.28	0.34	0.63	0.30

H_{cross} and S_{cross}, calculated considering that the interaction of all groups with water in the mixed surfactant system does not change with respect to that of Triton X-100:

$$H_{\text{cross}} = 2H_{\text{eff}}^{\text{w}} - 610.58, \tag{9}$$

$$S_{\text{cross}} = 2S_{\text{eff}}^{\text{w}} - 1.57. \tag{10}$$

The results obtained in the calculations of the fraction of cross-linking using CP measurements suggest that there is an important contribution of these reactions on the phase behaviour (Table 2). Therefore, the decrease observed in CP with an increase of the absorbed dose can be closely related to an increase in the occurrence of cross-linking reactions, even when mean distribution values of EO groups show less marked effects.

It is interesting to note that just a small amount of cross-linking can have a significant influence on CP values. In general, changes in CP upon irradiation of surfactant aqueous solutions above CMC are dominated by variations in the mean distribution value of EO groups and the formation of mixed micelles between Triton X-100 molecules and cross-linked species, where the second process seems to have a greater influence. After a certain dose, further changes in the cross-linking fraction seem to have a small effect on the macroscopic properties of Triton X-100, since micelles reach an interaction value which is very close to that of cross-linked surfactants. It is also important to note that calculated values (x_3) in this region should not be used as absolute numerical values, but rather as an indication of the extent of occurrence of cross-linking reactions.

3.2. Effect of gamma radiation on the CMC

Critical micelle concentration (CMC), similarly to CP, depends on the fine balance between hydrophobic and hydrophilic interactions [33]. Those surfactants with large heads in their chemical structure show a higher value of CMC, while those with bigger tails are characterized by smaller values. However, even when CP and CMC follow the same trend when the surfactant structure changes, the effect of these changes on the value of these macroscopic quantities is different.

The CMC experimental results of irradiated surfactants resemble those found for CP (Fig. 8). There is a significant decrease up to an absorbed dose of 50 kGy, and at higher doses, a slight increase of the CMC is observed.

Similarly to CP analysis, changes in CMC values can not be explained only using the variation in the mean distribution value of EO groups. Since the change in CMC for a series of monodisperse *p-t*-octylphenol-(n_e)-polyethoxylated surfactants is very small (ΔCMC = 0.2 mmol/L, for $\Delta n_e = 8$) [34], the decrease

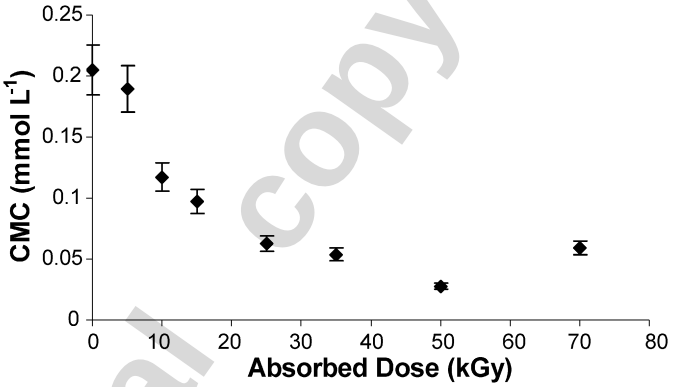

Fig. 8. Effect of gamma radiation on CMC of micellar solutions of Triton X-100 (1 wt%).

in the mean value of EO groups obtained using mass spectrometry can not explain the 8-fold decrease observed in CMC, as the absorbed dose increased. Nevertheless, we still may use the model of mixed surfactants to explain these results, in which case, the CMC of mixed surfactants can be obtained as if there is no interaction between surfactants [27].

$$C_{12}^{\text{M}} = \frac{C_1^{\text{M}} \cdot C_2^{\text{M}}}{C_1^{\text{M}}(1 - x_3) + C_2^{\text{M}} x_3}, \tag{11}$$

where C_{12}^{M}, C_1^{M}, C_2^{M} are the CMC of the mixed surfactants, the cross-linked surfactant and the Triton X-100 respectively; and x_3 is the cross-linking fraction. However, to obtain a complete description of C_{12}^{M} as a function of x_3, the CMC of both surfactants should be known. Since there is a small variation in CMC of Triton X-100 due to changes in EO number [34], we can consider C_2^{M} as approximately equal to the CMC of Triton X-100 without irradiation (obtained by experimental determination). In the case of the cross-linked surfactants, the C_1^{M} value must be estimated because it is not possible to perform measurements of the isolated species.

Using C_2^{M} and x_3 at a certain dose (e.g., at $D_{\text{abs}} = 15$ kGy, which is far below from the saturation region, $x_3 = 0.14$), the CMC calculated for cross-linked surfactants is $C_1^{\text{M}} = 2.23 \times 10^{-5}$ mol/L. This value is ten times smaller than the experimental value of the micellar Triton X-100 solution at the selected dose.

The fractions of cross-linking estimated using CP and CMC can be compared at the corresponding doses (Table 2). The good agreement of these results, up to a dose of 50 kGy, gives a strong support to the model of mixed surfactants, indicating the important effect that surfactants produced in cross-linking reactions can play in the variations of physicochemical properties of irradiated micellar solutions. The results obtained at high doses (>50 kGy) can be explained considering that in this

region, further changes in the fraction of cross-linking have little effect on the macroscopic quantities that are measured. The saturation zone predicted using both methods agreed, and can explain the small changes in the values obtained for CP and CMC when at absorbed doses of 50 kGy and higher.

3.3. Effect of gamma radiation on efficiency and effectiveness of surfactant adsorption

There are two important parameters that characterize the performance of a surfactant in lowering the surface tension of a solution. These are usually referred as the surface excess concentration at surface saturation, Γ_m (effectiveness of adsorption), and the surfactant concentration in the bulk phase required to produce a 20 N/m reduction in the interfacial tension of the solvent, $-\log C_{(-\Delta\gamma=20)} = pC_{20}$ (surfactant efficiency) [34]. The effectiveness of adsorption is an important factor in determining such properties as foaming, wetting, and emulsification, while the efficiency indicates the maximum reduction in surface tension that can be obtained regardless of the concentration.

The calculated values for these parameters using surface tension measurements of Triton X-100 micellar solutions showed a small increase in the surfactant effectiveness from 3.4 to 5.1 ($\times 10^{-6}$ mol/m^2). For surfactants with a single hydrophilic group, the area occupied by a surfactant molecule at the surface appears to be determined by the area occupied by the hydrated hydrophilic group rather than by the hydrophobic portion [21]. In POE surfactants, the hydrophilic portion is immersed in the aqueous phase in the form of a coil, and the cross-sectional area increases with the number of OE units [21]. The increase in effectiveness obtained in surfactant solutions after gamma irradiation is thus consistent with the decrease by one EO group as average observed in mass spectra. It is also interesting to note that the overall increase is higher than the expected value considering reported effectiveness of monodisperse Triton X-100 surfactants ($\Gamma_m = 2.5$ for $n_e = 9$, $\Gamma_m = 2.6$ for $n_e = 8$) [21]. This result corroborates the larger influence of cross-linking on the physicochemical properties, compared to changes in EO polymeric distribution. In this case, it can be understood considering that in the cross-linked species, the hydrophilic portion is increased by forming a new surfactant molecule, in a closer packing arrangement at the surface, compared to two single molecules of Triton X-100.

On the other hand, there is an increment in efficiency (pC_{20}) from 5.4 (non-irradiated sample) to values ranging between 5.5 and 7.13 (irradiated samples). The larger pC_{20}, the more efficiently the surfactant is adsorbed at the interface and the more efficiently it reduces surface tension. In POE nonionics, an increase in the number of EO units in the hydrophilic group, in contrast to its large effect in decreasing the effectiveness of adsorption, seems to cause only a small decrease in the efficiency of adsorption, which is rather dominated by the structure of the hydrophobic tail [21]. The cross-linked species have a hydrophobic portion which is twice as bigger as those of Triton X-100, but the action of these big structures are somewhat diminished since the hydrophilic groups are at a non-terminal position. In this case, the hydrophobic group acts as if it were branched at the position of the hydrophilic group, with the carbon atoms on the shorter portion of the hydrophobic group having about two-thirds the effect of the carbon atoms in the longer portion [21]. The overall result is an effective increase of the hydrophobic portion of the cross-linked species, with a consequent increasing effect of the surfactant efficiency.

In summary, both effectiveness and efficiency of Triton X-100 solutions increase after irradiation, which suggests that the surfactant essential function reducing protein surface adsorption is not affected. However, these parameters are mostly related to the surface activity of the surfactant, but there are other mechanisms influencing protein solubilization in formulations. For example, it is well known that nonionic surfactants also bind weakly to proteins. Surfactant–protein interactions are hydrophobic in nature because proteins with more hydrophobicity bind more surfactants (Bam et al. [35]). The mechanism of protein–surfactant interactions depends on the nature and concentration of the surfactant in the solution bulk; e.g., the adsorption of insulin on plastic bags is strongly decreased by the addition of Triton X-100 surfactant to the formulation, since the surfactant reduces the protein's available hydrophobic surface by binding at the hydrophobic patches on the surface of the protein [35]. Therefore, specific studies considering the individual components used in a formulation should be done to ensure that the surfactant structural changes detected in this investigation do not interfere in the surfactant–protein interactions responsible for solubilization.

4. Summary

Gamma irradiation of aqueous solutions containing Triton X-100 at concentrations above CMC affects some of the surfactant physico-chemical parameters, in particular at the dose range that is characteristic for radiation sterilization. In this region, both the CP and the CMC values decrease as the absorbed dose increases, probably due to changes in the MMD of the surfactant and the occurrence of cross-linking reactions. These two factors are corroborated by mass spectrometry analysis and the appearance of turbidity in samples irradiated at doses higher than 15 kGy. The results obtained from theoretical calculations using the Flory–Huggins model suggest that even a small fraction of cross-linked species formed after irradiation has a significant effect on the fine hydrophilic/lipophilic balance that controls the physico-chemical behaviour of micellar solutions. The consequences of changes on this behaviour are essential to asses the performance of nonionic surfactants and to understand their role in the pharmaceutical formulations, e.g. for stability reasons.

Acknowledgment

We would like to thank Dr. John P. Lowe from the Department of Chemistry at Bath for ^{13}C NMR measurements and analysis.

Supplementary information

The online version of this article contains additional supplementary information.

Please visit DOI: 10.1016/j.jcis.2007.02.081.

References

[1] A. Wade, P.J. Weller (Eds.), Handbook of Pharmaceutical Excipients, American Phamaceutical Association, Pharmaceutical Press, 1994.
[2] M. Khossravi, Y.H. Kao, R.J. Mrsny, T.D. Sweeney, Pharm. Res. 19 (2002) 634.
[3] H. Tomioka, T. Yoneyama, K. Asano, T. Watanabe, H. Saito, Microbiol. Immunol. 27 (1983) 673.
[4] American Standard "Sterilization of health care products—Radiation sterilization," ANSI/AAM/ISO 11137-1994.
[5] T. Zaharescu, S. Jipal, B. Gigante, Polym. Bull. 57 (2006) 729.
[6] T. Arakawa, S.J. Pretrelski, W.C. Kenney, J.F. Carpenter, Adv. Drug Delivery Rev. 46 (2001) 307.
[7] K. Bogman, F. Erne-Brand, J. Alsenz, J. Drewe, J. Pharm. Sci. 92 (2003) 1250.
[8] J. Perkowski, J. Mayer, K. Lech, Fibres Textiles Eastern Europe 13 (2005) 81.
[9] E. Pelizzetti, C. Minero, V. Maurino, A. Sciafani, H. Hidaka, N. Serpone, Environ. Sci. Technol. 23 (1989) 1380.
[10] J. Perkowski, J. Mayer, J. Radioanal. Nucl. Chem. Art. 141 (1990) 271.
[11] J. Perkowski, J. Mayer, J. Radioanal. Nucl. Chem. Art. 157 (1992) 27.
[12] J. Perkowski, J. Mayer, J. Radioanal. Nucl. Chem. Lett. 188 (1994) 211.
[13] A. Henglein, Th. Proske, Macromol. Chem. 179 (1978) 2279.
[14] K. Bogman, F. Erne-Brand, J. Alsenz, J. Drewe, J. Pharm. Sci. 92 (6) (2003) 1250.
[15] E.V. Batrakova, H.Y. Han, V. Alakhov, D.W. Miller, A.V. Kabanov, Pharm. Res. 15 (1998) 850.
[16] E. Batrakova, S. Lee, S. Li, A. Venne, V. Alakhov, A. Kabanov, Pharm. Res. 16 (1999) 1373.
[17] W.J. Xia, H. Onyuksel, Pharm. Res. 17 (2000) 612.
[18] G.C. Na, B.O. Yuan, H.J. Stevens Jr., B.S. Weekley, N. Rajagopalan, Pharm. Res. 16 (4) (1999) 562.
[19] L.P. Stratton, A. Dong, M.C. Manning, J.F. Carpenter, J. Pharm. Sci. 86 (1997) 1006.
[20] M. Poss, T. Couch, A. Odufu, J. McCann, J. Mellon, B. Melnick, D. Jenke, J. Chromatogr. Sci. 41 (2003) 410.
[21] J.R. Milton, Surfactants and Interfacial Phenomena, Wiley, New York, 2004.
[22] J. Ross, J.P. Olivier, J. Phys. Chem. 63 (1959) 1671.
[23] B.L. Carvalho, G. Briganti, J. Phys. Chem. 93 (1989) 4283.
[24] L.A.M. Rupert, J. Colloid Interface Sci. 153 (1992) 92.
[25] T. Inoue, H. Ohmura, D. Murata, J. Colloid Interface Sci. 258 (2003) 374.
[26] A. Chatterjee, S.P. Moulik, S.K. Sanyal, B.K. Mishra, P.M. Puri, J. Phys. Chem. B 105 (2001) 12823.
[27] K. Holmberg, B. Jönsson, B. Kronberg, B. Lindman, Surfactants and Polymers in Aqueous Solution, Wiley, Chichester, 2002.
[28] A.M. Rothman, J. Chromatogr. 253 (1982) 283.
[29] (a) D.C. Schriewer, L. Li, Anal. Chem. 68 (1997) 4169;
(b) C.N. McEwen, C. Jackson, B.S. Larson, Int. J. Mass Spectrom. Ion Processes 160 (1997) 387;
(c) H.C.M. Byrd, C.N. McEwen, Anal. Chem. 72 (2000) 4568.
[30] S.J. Wetzel, C.M. Guttman, J.E. Girard, Polym. Mater. Sci. Eng. 88 (2003) 74.
[31] H.H. Jaffé, M. Orchin, Theory and Applications of Ultraviolet Spectroscopy, Wiley, New York, 1962, chap. 12.
[32] W.N. Maclay, J. Colloid Sci. 11 (1956) 272.
[33] R. Kjellander, J. Chem. Soc. Faraday Trans. 278 (1982) 2025.
[34] D. Myers, Surfaces, Interfaces, and Colloids: Principles and Applications, second ed., Wiley, New York, 1999.
[35] N.B. Bam, T.W. Randolph, J.L. Cleland, Pharm. Res. 12 (1) (1995) 2–11.