Binding studies of curcumin to polyvinyl alcohol/polyvinyl alcohol hydrogel and its delivery to liposomes

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Binding of curcumin, an antioxidant and anti-tumour agent from turmeric, with polyvinyl alcohol (PVA) has been characterized by monitoring changes in the absorption and the fluorescence spectra. The product of the number of binding sites in PVA and the binding constant (K) was determined to be 2.4×10^5 and 1.9×10^5 M^{-1} by optical absorption and fluorescence techniques respectively. The hydrogel of PVA, produced by γ-irradiation of aqueous solution, also could be loaded with curcumin to a maximum extent of 215 nmol/g. The binding constant of curcumin to PVA hydrogel and the number of binding sites were determined to be 2.6×10^4 M^{-1} and 2.5 respectively. Studies on the release of curcumin in liposomes indicated that hydrogel could be used as an effective vehicle for transferring curcumin to model lipid membranes, liposomes.

Keywords: Curcumin, drug delivery, hydrogel, liposomes, polyvinyl alcohol.

HYDROGELS are hydrophilic polymer networks having the capacity to absorb water, ranging from about twenty to thousand times their dry weight[1]. Hydrogels can be prepared by: (a) cross-linking of water-soluble polymers, (b) conversion of cross-linked hydrophobic polymers, or (c) conversion of hydrophobic polymers to hydrophilic polymers, followed by cross-linking to form networks. Hydrogels are widely used in areas, such as drug delivery, immobilization of enzymes, de-watering of protein, etc. As these have a marked cooling effect and can also release water at the desired site, they are often used on dehydrated wounds, such as minor burns, grazes and pressure sores[2]. Synthetic polymers, such as polyacrylic acid (PAA), polyacryl amide (PAAm), poly *n*-isopropyl acrylamide (PNIPAAm), polyvinyl pyrrolidone (PVP) and polyvinyl alcohol (PVA), form hydrogels[3–9]. Some of these hydrogels have a lower critical solution temperature (LCST), above which they release the imbibed water. These hydrogels can adsorb different drugs, depending on the nature of the drug, the polymer and the pore size. One of the important requirements for the drug delivery systems is to deliver the drug to a target tissue efficiently at a steady therapeutic concentration, with minimum exposure to non-target tissues. The rate of release of the drug into the external environment can be controlled, depending on the compatibility between the drug molecule and the external fluid, and cross-linking density of the hydrogel[10]. PVA, a biocompatible polymer, is used for making hydrogel dressings[11]. Aqueous PVA solution can be easily cross-linked by γ-radiation to produce hydrogels with different cross-linking densities. Many drugs can be incorporated in such hydrogel matrices for their controlled release.

Since ancient times, curcumin, a yellow poly phenolic pigment from turmeric, has been used for skin protection. It is applied, in combination with other ayurvedic formulations, for the treatment of several superficial skin infections and burns. Curcumin suppresses the growth of many bacteria. It is a good cleansing agent. Curcumin is used commercially in several skin-lotion formulations. Curcumin and its derivatives are also known to exhibit a wide range of pharmacological activities, including anti-inflammatory, antioxidant, anti-carcinogenic and anti-HIV protease activity[12–15]. It is a hydrophobic molecule, and is practically insoluble in aqueous medium. Because of its hydrophobic nature and poor solubility in water, its bioavailability, after oral administration, is inadequate and, therefore, needs a carrier vehicle to transport to the desired targets. A suitable delivery system, such as PVA hydrogel, needs to be employed to deliver curcumin to the required target for treatment.

With this aim, the loading of curcumin to PVA hydrogel, and its release from the hydrogel into phosphatidylcholine (PC) liposome, were studied. Further, its binding capacity with aqueous PVA solution and PVA hydrogel was determined by spectroscopic techniques.

Materials and method

PVA (mol. wt. ~125,000; S.D. Fine Chemicals) and spectrograde methanol (Sisco Research Laboratory), were used in the study. Curcumin, cholesterol, acrylamide and egg yolk phosphatidylcholine were from Sigma. Rest of the chemicals used were obtained from local market, and were of the highest purity available. Aqueous solutions

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were prepared, using water obtained from Millipore-Q water purification system, and were purged with nitrogen, wherever required, to minimize degradation. ^{60}Co γ-source, with a dose rate of 2.6 kGy/h, was used for irradiation of the polymer solution. Absorption and fluorescence spectra were recorded, using Spectroscan UV 2600-spectrophotometer and Hitachi F-4010 fluorimeter respectively.

Results and discussion

Binding of curcumin with PVA solution

Optical absorption method: Aqueous solution of curcumin (10 μM), containing 5% methanol, shows maximum absorption at 426 nm. On addition of PVA, in the concentration range of 10–100 μM, the absorption maximum was blue shifted from 426 to 420 nm, with simultaneous increase in absorbance at 420 nm (Figure 1). These changes in the absorption spectra indicate an interaction between the ground states of curcumin and PVA. The process of binding of curcumin to PVA can be represented by the following:

$$\text{Curcumin} + S \rightleftharpoons \text{Curcumin} : S, \tag{1}$$

where S is the binding site in the polymeric chain, and [S] is the product of the number of binding sites in the polymeric chain n, and [PVA], i.e. [S] = n[PVA].

The binding constant (K) for the above equilibrium is given by:

$$K = \frac{[\text{Curcumin} : S]}{[\text{Curcumin}][S]}. \tag{2}$$

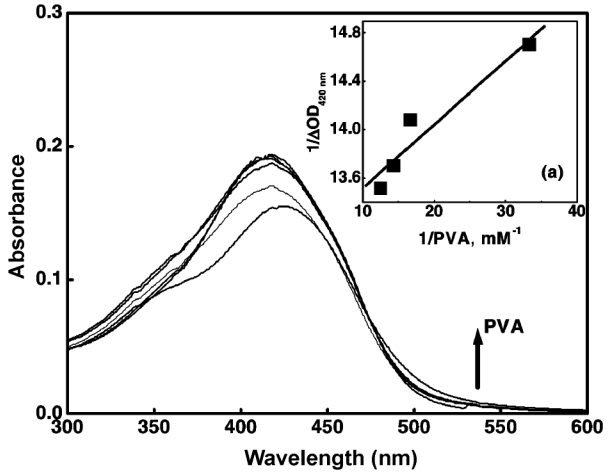

Figure 1. Absorption spectra of curcumin (10 μM) in aqueous solution in 5% methanol, in the absence and presence of varying concentrations of PVA (10–100 μM), at pH 7. Inset: Double reciprocal plot, in accordance with eq. (12).

Since [S] = n[PVA],

$$K = \frac{[\text{Curcumin} : S]}{n[\text{PVA}][\text{Curcumin}]}. \tag{3}$$

As optical absorption measurements were carried out in the presence of excess PVA, thus assuming n[PVA] > [Curcumin], the above equation can be written as,

$$K = \frac{[\text{Curcumin} : S]}{n[\text{PVA}]_0\,([\text{Curcumin}]_0 - [\text{Curcumin} : S])}. \tag{4}$$

Equation (4) is rearranged to give [Curcumin : S].

$$[\text{Curcumin} : S] = \frac{nK[\text{PVA}]_0\,[\text{Curcumin}]_0}{1 + nK[\text{PVA}]_0}. \tag{5}$$

The initial absorbance A_0, is due to curcumin and PVA. However, the final absorbance A_{eq} is the sum of absorbance due to complex, A_{eq}(Curcumin : S); free curcumin, A_{eq} (Curcumin), and PVA, A_{eq}(PVA).

$$A_{eq} = A_{eq}(\text{Curcumin} : S) + A_{eq}(\text{Curcumin}) + A_{eq}(\text{PVA}). \tag{6}$$

Differential absorption method (linear fitting): Since absorbance due to PVA at 420 nm is negligible, it can be neglected. Therefore, eq. (6) becomes

$$A_{eq} = A_{eq}\,(\text{Curcumin} : S) + A_{eq}\,(\text{Curcumin}). \tag{7}$$

Maximum absorption changes were seen at 420 nm; hence the binding constant was estimated by following the absorbance changes at the same wavelength, as a function of PVA concentration. Therefore, the change in absorbance at 420 nm can be given as:

$$\Delta A = A_{eq}(\text{Curcumin} : S) + A_{eq}\,(\text{Curcumin}) - A_0\,(\text{Curcumin}), \tag{8}$$

$$\Delta A = \varepsilon(\text{Curcumin} : S)l[\text{Curcumin} : S] + \varepsilon(\text{Curcumin})l \times [\text{Curcumin}] - \varepsilon(\text{Curcumin})l[\text{Curcumin}]_0, \tag{9}$$

$$\Delta A = \Delta\varepsilon l\,[\text{Curcumin} : S], \tag{10}$$

where l is the optical path length, which is 1 cm, and $\Delta\varepsilon$ corresponds to the differential extinction coefficient at 420 nm. From eqs (5) and (10), we get

$$\Delta A = \Delta\varepsilon\; l\frac{nK[\text{PVA}]_0\,[\text{Curcumin}]_0}{1 + nK[\text{PVA}]_0} \tag{11}$$

which is rearranged to give,

$$\frac{1}{\Delta A} = \frac{1}{nKl\Delta\varepsilon[\text{Curcumin}]_0}\left(\frac{1}{[\text{PVA}]_0}\right) + \frac{1}{\Delta\varepsilon l[\text{Curcumin}]_0}. \quad (12)$$

The absorbance values at the respective PVA concentrations were fitted to a double reciprocal plot (eq. 12), the modified Benesi–Hildebrand plot. Figure 1 (inset) shows the double reciprocal plot, $1/\Delta A$ vs $1/[\text{PVA}]_0$, and the linear fit. The slope and intercept of the plot are $1/(nK\Delta\varepsilon l[\text{Curcumin}]_0)$ and $1/(\Delta\varepsilon l[\text{Curcumin}]_0)$, respectively. The ratio of the intercept and the slope gives the product of the number of the binding sites and the binding constant (nK). The differential extinction coefficient was calculated from the reciprocal of the intercept. Under this condition, the product nK and the differential extinction coefficient estimated at 420 nm, were found to be $2.4 \pm 0.2 \times 10^5\ \text{M}^{-1}$ and $2.7 \pm 0.2 \times 10^4\ \text{M}^{-1}\ \text{cm}^{-1}$ respectively. Using the value of the extinction coefficient of free curcumin at 420 nm to be $4.88 \times 10^4\ \text{M}^{-1}\ \text{cm}^{-1}$, the extinction coefficient of PVA-bound curcumin was determined to be $7.58 \times 10^4\ \text{M}^{-1}\ \text{cm}^{-1}$.

Alternate absorption method (non-linear fitting): Nonlinear least-squares regression is an alternative approach of data analysis, which is used to fit the data directly into the relevant equations. For this approach, eq. (7) can be written as

$$A_{\text{eq}} = A(\text{Curcumin} : S)\frac{[\text{Curcumin} : S]}{[\text{Curcumin}]_0} + A_0\frac{[\text{Curcumin}]}{[\text{Curcumin}]_0}, \quad (13)$$

where $A(\text{Curcumin} : S)$ represents the absorbance at 420 nm at saturating concentration of PVA, and eq. (13) can be written as,

$$A_{\text{eq}} = A(\text{Curcumin} : S)\frac{[\text{Curcumin} : S]}{[\text{Curcumin}]_0} + A_0\frac{[\text{Curcumin}]_0 - [\text{Curcucmin} : S]}{[\text{Curcumin}]_0}, \quad (14)$$

$$\frac{[\text{Curcumin} : S]}{[\text{Curcumin}]_0} = \frac{A_0 - A_{\text{eq}}}{A_0 - A(\text{Curcumin} : S)}. \quad (15)$$

From eqs (5) and (15), after rearrangement, we get

$$A_{\text{eq}} = \frac{A_0 + A(\text{Curcumin} : S)\, nK[\text{PVA}]_0}{1 + nK[\text{PVA}]_0}. \quad (16)$$

The binding constant was evaluated using the nonlinear fitting, in accordance with eq. (16). The value of nK, determined by nonlinear least-squares fitting of the data (plot not shown), was found to be $2.1 \pm 0.1 \times 10^5\ \text{M}^{-1}$. The results obtained from both the linear and the nonlinear regression match reasonably well.

Fluorescence method: Curcumin exhibits medium-sensitive fluorescence properties. In an aqueous buffer, it exhibits a broad, weak fluorescence band, with maximum at ~550 nm, but in a hydrophobic environment, its fluorescence intensity is found to increase, with a blue shift in the fluorescence maximum. This photophysical property of curcumin was utilized to estimate the mode of binding and the binding constant of curcumin with PVA. For this study, curcumin was excited at 380 nm, as the absorption intensity at this wavelength does not change much with PVA concentration. Figure 2 shows that the fluorescence intensity of curcumin increases significantly in presence of PVA, along with a blue shift in the fluorescence maximum. In the presence of the highest studied concentration of 100 μM PVA, the fluorescence maximum shifted towards the blue region by ~20 nm, from a broad, weak band ($\lambda_{\text{max}}^{\text{em}} \sim 550$ nm) to a well-defined fluorescence band ($\lambda_{\text{max}}^{\text{em}} \sim 530$ nm). The observed blue shift in the fluorescence spectrum indicates probable binding of curcumin to the hydrophobic pockets of PVA. Similar to the case of absorbance, eq. (16), the binding constant K is related to the fluorescence intensities as given below[16].

$$F_{\text{eq}} = \frac{F_0 + F(\text{Curcumin} : S)\, nK[\text{PVA}]_0}{1 + nK[\text{PVA}]_0}, \quad (17)$$

where F_0 and F_{eq} are the respective fluorescence intensities from curcumin at 530 nm, in the absence and the presence of different concentrations of PVA (10–100 μM),

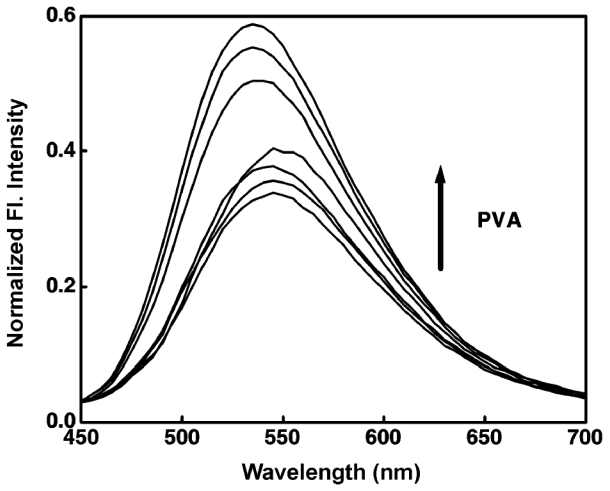

Figure 2. Fluorescence spectra of curcumin (10 μM) in aqueous solution, containing 5% methanol, in the absence and presence of varying concentrations of PVA (10–100 μM), at pH 7. The samples were excited at 370 nm.

and F(Curcumin : S) is the saturating fluorescence intensity of curcumin in the presence of the highest PVA concentration. Nonlinear least-square fitting of the data (plot not shown) using eq. (17), gave nK as $1.9 \pm 0.1 \times 10^5\ M^{-1}$, which is slightly lower than the value obtained by the absorption methods.

Nature of binding sites in PVA

Quenching studies of fluorescence intensity of curcumin bound to PVA were carried out using iodide and acrylamide as quenchers. These studies were carried out to evaluate the location and nature of the curcumin-binding sites inside PVA chains. Iodide is a hydrophilic quencher that can access curcumin bound in the hydrophilic part of PVA, while acrylamide, being hydrophobic, can access curcumin only when inserted inside the hydrophobic part of PVA. The concentrations of the quenchers were varied from 0 to 0.3 M, keeping the ionic strength constant at 0.3 M. The fluorescence data were analysed according to the Stern–Volmer equation:

$$\frac{F_0}{F} = 1 + K_d[\mathrm{Q}], \tag{18}$$

where F_0 and F are the intensities of the fluorescence (curcumin bound to PVA) in the absence and the presence of the quencher Q respectively, and K_d is the Stern–Volmer collision constant. Figure 3 (inset a and b) shows the Stern–Volmer plot obtained by monitoring quenching of curcumin fluorescence at 420 nm, in the presence of iodide and acrylamide quenchers respectively. The Stern–Volmer plots for both the quenchers show deviation from linearity, indicating the presence of two types of fluorophores, one of which may not be easily accessible to the quencher[17].

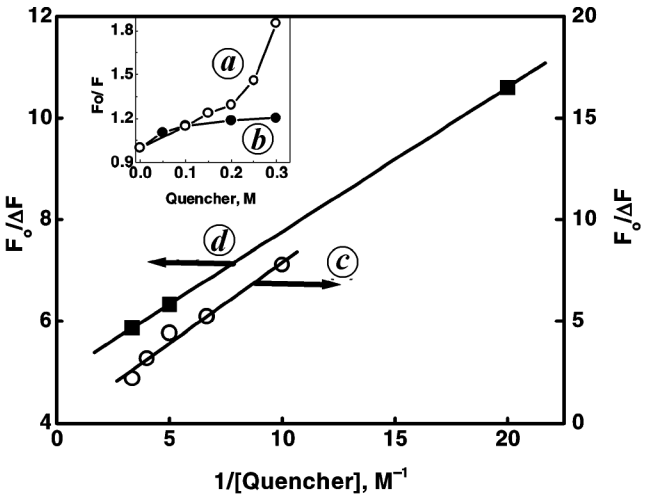

Figure 3. (Inset) Stern–Volmer plot for quenching of curcumin–PVA complex with iodide (*a*) and acrylamide (*b*) at pH 7, (*c*) and (*d*) represent modified Stern–Volmer plots obtained on quenching of curcumin–PVA complex with iodide and acrylamide respectively, at pH 7.

Curcumin has phenolic OH groups, which can be ionized, with $pK_a \sim 8$ and at pH 7, it has contributions from both the ionized and the non-ionized forms. It is possible to evaluate the fraction of curcumin located in the hydrophobic and hydrophilic pockets inside PVA, by following the quenching of fluorescence from PVA-bound curcumin, using acrylamide and iodide as quenchers at pH 7. For this, the curcumin–PVA solutions were incubated in quartz cells at 30°C, at different concentrations of iodide (I^-) and acrylamide as quenching agents, separately, in 10 mM phosphate buffer. The fluorescence intensity changes ($\Delta F = F_0 - F$) due to curcumin were treated with the modified Stern–Volmer equation, according to the procedure given in Kunwar *et al.*[18]:

$$\frac{F_0}{\Delta F} = \left(\frac{1}{f_a K'_d[Q]}\right) + \left(\frac{1}{f_a}\right), \tag{19}$$

where F_0 and ΔF are the fluorescence intensity in the absence and change in fluorescence in the presence of the quencher Q respectively, f_a is the fraction of the initial fluorophore that is accessible to the quencher and K'_d is the modified Stern–Volmer collision constant, which measures the stability of the quencher–probe complex, and is related to the separation distance in the excited state complex. Experimental results are plotted in Figure 3 *c* for I^-, and in Figure 3 *d* for acrylamide titrations. On fitting the data obtained from quenching in the presence of iodide to eq. (19), we found $f_a = 0.25 \pm 0.01$ and $K'_d = 19.1 \pm 1.7\ M^{-1}$ at pH 7. Similarly, fitting the data obtained from quenching in the presence of acrylamide to eq. (19), f_a value of 0.20×0.01 and K'_d value of $17.3 \pm 2.11\ M^{-1}$ were determined at pH 7. The sum of the fractions of the curcumin embedded in hydrophilic and hydrophobic cavities does not equate to one, which indicates that curcumin may not be uniformly present in the gel, and some fraction of the curcumin in the hydrophobic interior is not accessible to acrylamide[17]. The above two sets of experiments confirmed that at pH 7, which is close to physiological pH, curcumin is embedded in both hydrophobic and hydrophilic sites of PVA. Whereas the hydrophobic nature of curcumin and the observed blue shift in the fluorescence spectrum in the presence of PVA indicate preferential binding of curcumin to hydrophobic pockets of PVA. Therefore, it may be concluded that hydrophobic sites of PVA chains are mainly responsible for binding of curcumin, along with some contribution from the hydrophilic sites.

Binding of curcumin with PVA hydrogel

Aqueous PVA solution as such cannot be used for controlled drug delivery. It has to be cross-linked either by γ-

radiation method or by a chemical method to form hydrogel. Several reports have shown the use of hydrogel as an efficient drug-delivery agent[19–22]. Hydrogels have the ability to absorb solutes, or drugs, from solutions and can release them in a controlled manner in a suitable medium, to have application as drug-delivery systems. Therefore, binding of curcumin to PVA hydrogel, produced by γ-ray induced cross-linking method, was also studied.

For this purpose, hydrogel was prepared by exposing 5 ml of 5% aqueous PVA solution to a total γ-radiation dose of ~13 kGy, in a closed container of cross-sectional area 1×1 cm^2. The PVA gel samples were washed with nanopure water to remove uncross-linked polymer fraction, and kept in nanopure water. A known weight (~1 g) of the swollen gel was immersed in 10 ml solution of known concentration of curcumin (typically around 20–250 μM), dissolved in 5% methanol–water (v/v) mixture. It was incubated for nearly 8 h. The loading of curcumin into the hydrogel was monitored by recording the absorption spectrum of the supernatant solution as a function of time. Figure 4 shows the absorption spectra of the supernatant solution, with initial concentration of 150 μM curcumin for different incubation times. From the absorption spectra, it is clear that the absorbance in the methanol–water solution at 426 nm decreases with time, indicating loading of curcumin into the hydrogel. After 8 h, there was not much change in the absorption spectrum, indicating an equilibrium state. The amount of curcumin loaded into the hydrogel was calculated from the difference in absorbance of the curcumin solution at 426 nm, before and after incubating with hydrogel, and using the extinction coefficient value of 4.88×10^4 M^{-1} cm^{-1}. Thus, on incubating hydrogel with different concentrations of curcumin (20–250 μM), the amount of curcumin loaded was estimated to be in the range 20–215 nmol/g of the hydrogels. Figure 4 (inset, a) shows the amount of curcumin adsorbed in one gram of hydrogel as a function of the initial curcumin concentration. The amount of curcumin loaded in the hydrogel was found to increase with increasing concentration of curcumin in the solution. Figure 4 (inset, b) also shows the number of nanomoles of curcumin adsorbed from 100 and 150 μM curcumin solution by one gram of hydrogel as a function of time.

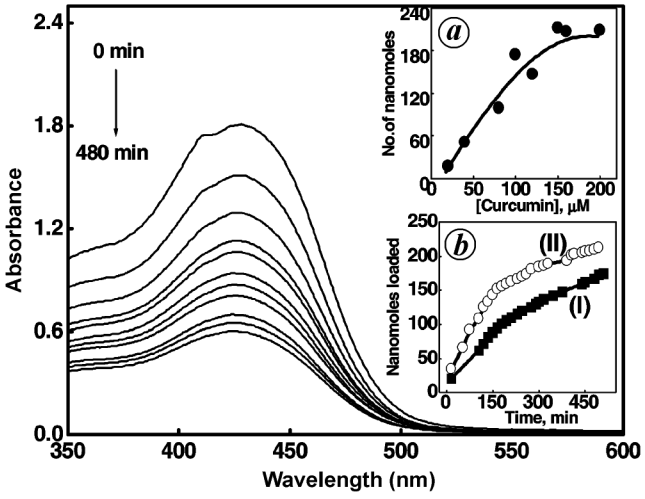

Figure 4. Absorption spectra of supernatant solution at different times, at an interval of 30 min (with initial curcumin concentration of 150 μM). Inset: ***a***, Nanomoles of curcumin adsorbed per gram of hydrogel as a function of curcumin concentration at saturation stage (incubation time 8 h). ***b***, Number of nanomoles of curcumin adsorbed per gram of hydrogel, as a function of time, with initial curcumin concentration of (I) 100 μM and (II) 150 μM.

At equilibrium, the concentration of curcumin in the solution is given by the equation:

$$k_b(1 - \theta)[\text{Curcumin}] = k_d\theta, \tag{20}$$

where k_b and k_d are rate coefficients of the binding and dissociation reactions respectively, and θ is the fraction of the binding sites occupied at equilibrium.

$$\theta = \frac{\text{Moles of curcumin bound}}{\text{Moles of binding sites}}$$

$$= \frac{\text{Moles of curcumin bound}}{n \times \text{Moles of PVA in the gel}},$$

where n is the number of the binding sites in a polymer chain. Equation (20) can be rearranged to give the Langmuir equation[23]:

$$r = \frac{nK[\text{Curcumin}]}{1 + K[\text{Curcumin}]}, \tag{21}$$

where K (k_b/k_d) is the binding constant and r (= θn) is the binding ratio which is defined as the 'ratio of the moles of curcumin bound with hydrogel to the moles of PVA'. To get the best values for binding parameters, linearization methods of eq. (21) developed by Klotz has been used.

Reciprocal of eq. (21) gives the Klotz plot, i.e.

$$\frac{1}{r} = \frac{1}{n} + \frac{1}{nK[\text{Curcumin}]} \tag{22}$$

The plot of $1/r$ vs $1/[\text{Curcumin}]$, as shown in Figure 5, gives a straight line, with slope $1/nK$ and intercept $1/n$. The binding constant was found to be 2.6×10^4 M^{-1} and the number of binding sites was 2.5 per polymer chain. The nK value of the PVA hydrogel (6.5×10^4 M^{-1}) was lower than that of aqueous PVA. This difference could be due to lower value of n or K, or both, in the PVA hydrogel because of configurational constraints. Figure 6 shows a representative piece of PVA hydrogel loaded with curcumin. Uniform loading of curcumin throughout the hydrogel is clearly visible.

Release of curcumin from hydrogel into liposomes

To utilize biocompatible hydrogel for delivery of curcumin into a targetted bio-system, it is important to study its releasing characteristics from the curcumin–hydrogel system into a suitable medium. It is also important to check whether curcumin is released into water from the hydrogel. Therefore, a known weight (~0.2 g) of curcumin-loaded PVA hydrogel was incubated with 5 ml of water. The solution was checked spectrophotometrically for curcumin release, by monitoring the absorbance at 426 nm at different time intervals up to 5 h. No trace of curcumin was observed in the water. The same procedure was repeated with 5 and 10% methanol–water mixtures, and even in these solvent mixtures, no release of curcumin was observed. Further, increase in methanol concentration resulted in hydrogel deformation. Therefore, higher concentration of methanol was not tested for release of curcumin.

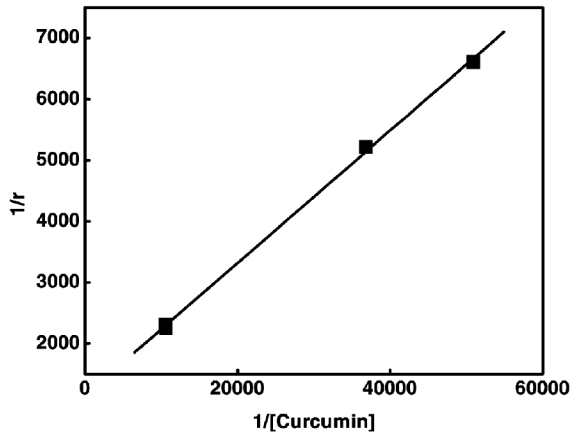

Figure 5. Linear Klotz plot for binding of curcumin to PVA hydrogel.

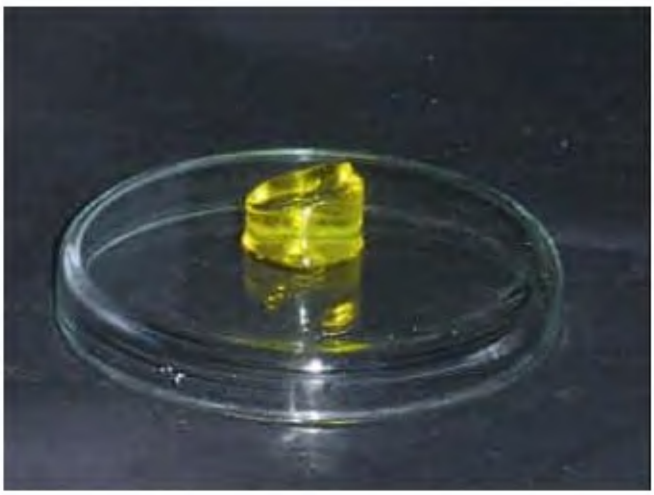

Figure 6. Uniformly loaded PVA hydrogel with curcumin.

Liposomes, having a bilayer lipid-like structure, are being used as models for cell membrane. The phosphatidylcholine lipid, in the presence of water, arranges itself to form liposome. Therefore, release of curcumin from the PVA hydrogel into the liposomal solution was used to evaluate its releasing capacity. In an earlier work from our laboratory[18], the binding constant of curcumin to PC liposome was found to be in the order of 10^4 to 10^5 M^{-1}. Since in this work the binding constant of curcumin to the PVA hydrogel has been estimated to be in the order of 10^4 M^{-1}, one could expect its release in the presence of PC liposome. Therefore, the release of curcumin from hydrogel was studied in the presence of different concentrations of liposome, ranging from 0.5 to 1.5 mg/ml.

Liposomes were prepared by the reported procedure[18]. Briefly, phosphatidylcholine and cholesterol were dissolved in chloroform in the weight ratio of 2 : 1, followed by solvent evaporation in rotavapour. The resulting thin film was solubilized in 10 mM phosphate buffer (pH 7.4), and sonicated for 5 min, using a bath type sonicator. The concentration of the phospholipids in liposomal solution was determined according to the method reported earlier.

The curcumin-loaded hydrogel samples were kept in 5 ml of 0.5–1.5 mg/ml liposome solution, and the samples were incubated at 37°C, using a thermostated waterbath. After 15 min intervals, the solution was checked spectrophotometrically for curcumin release, by monitoring the absorbance at 426 nm. Figure 7 shows the percentage of curcumin released, on incubation of liposome solutions with curcumin-loaded hydrogel. The progress of the release of curcumin was monitored as a function of time up to 5 h. It can be seen from Figure 7 that in 0.5 mg/ml of liposome, only about 40% of curcumin is

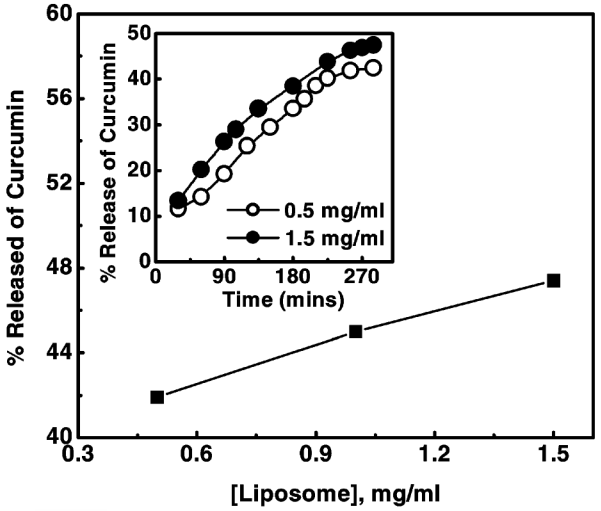

Figure 7. Plot of percentage of curcumin released (from hydrogel loaded with 250 μM curcumin solution) as a function of liposome concentration at equilibrium. (Inset) Percentage of curcumin released in 0.5 and 1.5 mg/ml liposome solution from the curcumin-loaded hydrogel obtained by equilibrating with 250 μM curcumin solution as function of time.

released in 4½ h. But, at higher concentration, 1.5 mg/ml of liposome, almost 50% of curcumin is released in the same time-period.

The release of curcumin in the liposome was also studied at different concentrations of curcumin loaded in 1 g of hydrogel. For this, hydrogels, containing varying amounts of curcumin, ranging from 20 to 215 nmol of curcumin per gram of hydrogel, were incubated with liposome solution. It was observed that with the increase of curcumin in the hydrogel, more curcumin was released in the liposome.

Conclusion

We have studied the binding of curcumin to PVA by absorption and fluorescence techniques. The product nK and the differential extinction coefficient were determined to be $2.4 \times 10^5\ M^{-1}$ and $2.7 \times 10^4\ M^{-1}\ cm^{-1}$ respectively, by absorption technique, whereas the nK was found to be $1.9 \pm 0.1 \times 10^5\ M^{-1}$ by the fluorescence technique. Quenching studies by hydrophobic and hydrophilic quenchers showed that at pH 7, curcumin is in both the hydrophobic and hydrophilic pockets of PVA. Aqueous PVA solution could be easily cross-linked to form hydrogel and loaded with curcumin. The results show that with increase in the concentration of curcumin, more curcumin gets loaded in the hydrogel. The rate of release of curcumin from the hydrogel can be easily controlled by factors such as initial concentration of curcumin in the hydrogel, concentration of liposome in the release medium, etc. The release of curcumin from hydrogel to liposome may take place by passive diffusion when there is contact between hydrogel and liposome. Thus, it has been established that PVA hydrogel could be applied as a drug-delivery system for controlled release of curcumin. This study is a model for future biological applications of curcumin hydrogel.

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