*Latin American Journal of Pharmacy*
(formerly *Acta Farmacéutica Bonaerense)*
*Lat. Am. J. Pharm.* **28** (1): 19-26 (2009)

Original Article

Received: September 24, 2008
Accepted: October 27, 2008

# Impregnation of Chitosan Microspheres with the Natural Dye Curcuma

Alexandre L. PARIZE [1], Melina HELLER [2], Valfredo T. FÁVERE [1], Mauro C.M. LARANJEIRA [1], Inês M.C. BRIGHENTE [1], Gustavo A. MICKE [2] & Tereza C.R. SOUZA [1]*

[1] *Laboratório QUITECH, Universidade Federal de Santa Catarina, Campus Trindade, 88040-900, Florianópolis, SC – Brazil.*

[2] *LABEC, Universidade Federal de Santa Catarina, Campus Trindade, 88040-900, Florianópolis, SC – Brazil.*

***SUMMARY.*** **The purpose of this study was to investigate the impregnation of chitosan microspheres with the natural dye curcuma. The impregnation with curcuma dye was investigated in aqueous medium at pH 9.0, 9.5 and 10.0. The process of impregnation was monitored using capillary electrophoresis analysis which was carried out to observe the presence of dye in the impregnated microspheres. The microspheres loaded with dye at pH 10.0 were evaluated by infrared spectroscopy, optical microscopy, scanning electron microscopy and thermal analysis. The dye was impregnated in the chitosan microspheres through an adsorption process and was released when placed in contact with acidic solutions at pH 1.0–5.0. The dye was released from the chitosan in less than 3 h, regardless of the pH, although most of the microspheres dissolved within 1 h. The release mechanism followed the Super Case II transport release model.**

## INTRODUCTION

The major pigment in commercial turmeric rhizome extracts (*Curcuma Longa* L.) is curcumin. Curcumin (1,7-bis (4-hydroxy-3-methoxy phenyl)-1,6-heptadione-3,5-dione) is a yellow pigment and is widely used in the food industry as a condiment [1] (Fig. 1). In recent years, the use of natural dyes has steadily increased, due to changes in consumer preference toward natural products having functional properties [1,2]. Over the past three decades this natural dye has been the subject of hundreds of publications focusing on its applications, for example, as an antioxidant, an anti-inflammatory, in the treatment of cardiovascular and arthritic diseases [3–6], as a cancer chemopreventive and chemo-therapeutic agent [6–9], as an anti-septic, and for wound healing and tissue protection [10,11]. Due to these properties, this dye can be used as a nutraceutical tool, acting in the prevention of some diseases.

It has been observed that curcumin has a poor solubility in water and acid solutions and a good solubility in basic medium and in any solvents, such as ethanol, acetone and dimethylsulphoxide [6]. At alkaline pH in the presence of light, a rapid degradation and instability of pure curcumin has been observed [12–14]. Thus, studies have been carried out to improve the solubility of pure curcumin through chemical modification or derivatization [15], and complexation or interaction with macromolecules [16]. But in some cases, slow complexation processes and the pH of the medium may limit its practical utility [17]. Some studies have focused on the interactions between curcumin, surfactants and amphiphilic molecules [18], forming micelles which are widely used in the cosmetics and pharmaceutical industries. Recently, some researches have used the impregnation of polymer surfaces with curcumin for several applications [19,20].

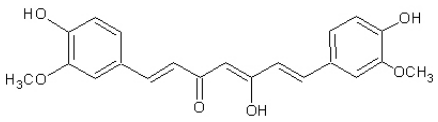


**Figure 1**. Chemical structure of curcumin.

*KEY WORDS:* Chitosan, Controlled Release, Curcuma Dye, Impregnation, Microencapsulation.

* Author to whom correspondence should be addressed. *E-mail:* tereza@qmc.ufsc.br

In relation to the association between curcumin and macromolecules, little information can be found on the use of the biopolymer chitosan [21]. Chitosan is a copolymer formed by units of 2-deoxy-N-acetyl-D-glucosamine and 2-deoxy-D-glucosamine joined through glycoside β(1→4) bonds, obtained from the alkaline deacetylation of chitin [22]. Due to the nature of its chemical configuration and its abundance, non-toxicity, hydrophilicity, and anti-bacterial properties, it has been employed in the preparation of films, gels and spheres, for a great variety of applications [23]. A factor which makes chitosan highly attractive is that it represents a renewable resource [24,25].

In this study, we impregnated chitosan microspheres with the natural dye curcuma in order to obtain a sample with potential uses in the food and pharmaceutical industries, guaranteeing the preservation of the dye properties and aiming at improving its solubility.

## MATERIALS AND METHODS

### *Materials*

Chitosan (molecular weight around 122.7 x $10^3$ g.mol$^{-1}$; degree of deacetylation of 86%) was purchased from Purifarma, Brazil. The natural dye curcuma (T-5-WS-P) was kindly supplied by Christian-Hansen Ind. & Com. Ltd. Pure standard curcumin was acquired from Merck. All other reagents employed were of analytical grade.

### *Preparation of chitosan microspheres*

Chitosan microspheres were prepared through the phase inversion or coacervation method. Chitosan was dissolved in 5% (v/v) acetic acid to produce a viscous 3% (w/v) chitosan solution and subsequently transferred with a nozzle to a bath containing a 2.0 mol L$^{-1}$ NaOH solution through an Ismatec peristaltic pump. After a contact time of 30 min the microspheres were filtered, washed at pH 7.0, and vacuum dried.

### *Effect of pH on adsorption*

The chitosan microspheres (150 mg) were placed in contact with 25.0 mL of a solution of the dye in an initial concentration of 400 mg.L$^{-1}$ in a pH range of 6.0 to 12.0 using phosphate buffer solutions (pH 6.0-8.0) and $NH_4Cl/NH_4OH$ (pH 9.0-12.0). The solutions containing the dye, after contact with the microspheres, were transferred to a Mini Shaker Marconi incubator, model MA 832, where they remained under stirring for 2 hours at 25.0 ± 0.1 °C. The supernatant of each flask was separated by filtration and the concentration of dye determined by UV-Vis spectroscopy using a curcumin standard calibration curve. The amount of dye adsorbed was calculated through Equation [**1**]:

$$q = \frac{C_o - C_{eq}}{W} \cdot V \qquad [\mathbf{1}]$$

where $q$ is the amount of dye adsorbed on the polymer (mg/g), $V$ is the volume of the solution (L), $W$ is the mass of the chitosan microspheres used (g), and $C_o$ and $C_{eq}$ are the initial concentration and the equilibrium concentration of dye in the solution (mg.L$^{-1}$).

### *Impregnation of chitosan microspheres with the natural dye curcuma*

The chitosan microspheres were loaded with dye employing an impregnation method. This method consisted of the dissolution of 500 mg of dye in buffer solutions NH4OH/NH4Cl pH 9.0, 9.5 and 10.0, respectively, (from a previous study) and the resulting solutions were filtered to remove any impurities. These solutions were then placed in contact with 750 mg of microspheres for 6 h at 25 °C, under continuous stirring and protected from light. The microspheres were filtered and washed with ethanol to remove the excess dye from the microsphere surface. The impregnated microspheres were left to dry at room temperature.

### *Characterization by infrared spectroscopy*

FTIR spectra were obtained for the pulverized form of the original chitosan and pigment-loaded microspheres with a spectrophotometer (Perkin Elmer, Model FT-PC-16). The samples were prepared as KBr pellets.

### *Morphology of the microspheres*

In order to study the shape, size and external morphology of the microspheres, samples were analyzed by scanning electron microscopy (SEM). The microsphere samples were placed on stubs and covered with a layer of gold/palladium to form a conductive film using a Cool Sputter Coater from Bal-tec, SDC 005. Micrographs of the samples were then obtained using a scanning electron microscope (Philips, Model XL 30) at an intensity of 10 kv, using various magnifications.

***Optical microscopy***

The impregnated layer of dye was evaluated using a Leica optical microscope, model DM 4000M, in the reflection mode. Photographs were obtained by instantaneous digitalization by means of a digital camera, model DC 300.

***Capillary Electrophoretic analysis***

All experiments were performed on an Agilent Technologies HP$^{3D}$CE apparatus (Palo Alto, CA, U.S.A.), equipped with a diode array detector. Data acquisition and treatment were performed with HP Chemstation software. This analysis was carried out following the method reported by Yuan and collaborators [26].

The chitosan microspheres (30 mg) impregnated in buffer solution at pH 9.0, 9.5 and 10.0 were dissolved in 4 mL of 100 mmol.L$^{-1}$ sodium acetate buffer by sonication for 30 min to extract the microsphere content, followed by addition of 6 mL of acetonitrile. The samples were then centrifuged and the resultant solution was collected and analyzed.

Electropherograms were recorded as follows: standard solutions and samples were introduced from the outlet and injected hydrodynamically at 50 mbar (5.25 Pa) for 9 s with reverse pressure. UV detection was performed at 360 and 429 nm. The applied separation voltage was 30 kV positive polarity on the injection side. The electrolyte was composed of sodium tetraborate (STB) and 20.0x10$^{-3}$ mol L$^{-1}$. At the start of each new working session, the capillary was conditioned at 25 °C and flushed with 1.0 mol L$^{-1}$ sodium hydroxide for 10 min, followed by deionized water for 5 min and finally with the background electrolyte (BGE) for 10 min. Between runs with the same buffer, the capillary was rinsed for 0.5 min with BGE. At the end of the analysis, the capillary was rinsed for 5 min with 1.0 mol L$^{-1}$ sodium hydroxide and for 10 min with deionized water.

***Differential scanning calorimetry***

Thermograms of the curcuma dye, original chitosan and dye-loaded microspheres were obtained with a differential scanning calorimeter (Shimadzu-DSC50) interfaced to a computer. Samples (12 mg) were sealed in aluminum pans. The scanning rate throughout the study was 10 °C.min$^{-1}$ and a dynamic $N_2$ atmosphere was maintained using a flow rate of 50 mL min$^{-1}$. All tests were carried out in duplicate.

***Swelling degree***

The dry microspheres were initially weighed and then kept in the different buffer solutions: pH 1.0 and pH 2.0 (HCl 0.1 and 0.01 mol.L$^{-1}$); pH 3.0, pH 4.0 and 5.0 ($CH_3COOH$ 0.1 mol.L$^{-1}$). The solutions containing the microspheres were transferred to a Mini Shaker Marconi incubator, model MA 832, and they were maintained under stirring at 25.0 ± 0.1 °C. At predetermined time intervals the microspheres were removed from the buffer solutions and dried with adsorbent paper to remove the excess of solution and weighed again.

The swelling capacity of the microspheres was calculated using Equation [**2**]:

$$swelling\ \frac{W_t - W_o}{W_o} \times 100 \qquad [\mathbf{2}]$$

where $W_t$ and $W_o$, refer to the weights of the samples at time $t$ and in the dry state, respectively. The microsphere swelling data are given as the mean of three determinations.

***Release studies***

Microsphere samples (25.0 mg) were suspended in 25.0 mL of buffer solution: pH 1.0, pH 2.0, pH 3.0, pH 4.0 and 5.0. At predetermined time intervals, 3 mL samples were removed to determine the quantity of dye released and then returned to the flask (25.0 ± 0.1 °C). The samples were analyzed at 429 nm, using a UV-Vis spectrophotometer. The percentage of dye released was calculated using the standard curcumin calibration curve (y = 0.0126 + 0.0794.x; $R^2$ = 0.9982). All the experiments were performed in triplicate. The results are expressed as a percentage of the dye released. The release study was conducted until the total dissolution of the microspheres.

**RESULTS AND DISCUSSION**

Figure 2 shows the amount of dye adsorbed in the chitosan microspheres as a function of pH. This study was carried out in the pH range of 6.0 – 12.0, since chitosan is soluble in acidic medium. Below pH 5.5 chitosan forms a gel and its capacity to adsorb dye cannot be evaluated [25]. The adsorption of the dye increases in alkaline medium and it was observed that the optimum adsorption pH was 11.0 (Fig. 2). Since pure curcumin may degrade at highly alkaline pH [12] the values of pH 9.0, 9.5 and 10.0 were selected for a more detailed study of the dye impregnation.

The process of dye impregnation at pH 9.0, 9.5 and 10.0 was monitored using capillary elec-

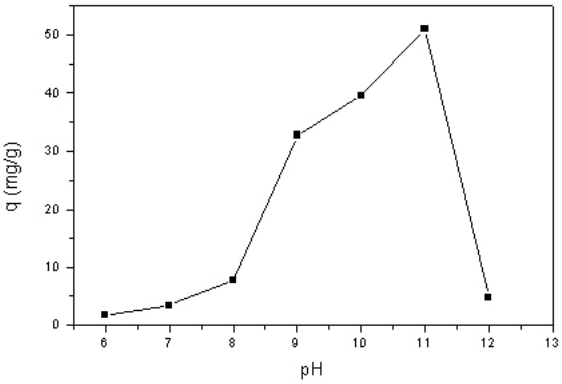


**Figure 2**. pH dependence for adsorption of natural curcumin dye at 25 ± 0.1 °C, $C_o$ = 400 mg.L$^{-1}$ with a contact time of 2 hours.

trophoresis analysis. This particular analysis was carried out to observe the presence of curcumin in the impregnated microspheres.

The capillary electrophoresis analysis shows that an increase in the pH of the impregnation solution favors the interaction between the dye and chitosan biopolymer (Fig. 3A). The pKa values for curcumin are 8.38, 9.88 and 10.51 [27]. Since curcumin is ionized at pH 9.0, 9.5 and 10.0 it is expected to have a higher affinity for chitosan through its amino and phenolate groups. Curcumin has been observed to have a strong affinity for amines and positively charged amino acids [28]. Figure 3A shows a quantitative increase in the concentration of dye in the microspheres with pH, observing a maximum at pH 10.0. On the other hand, it is also observed that vanillin is present in the microspheres (Fig. 3B), formed in solution due to the alkaline degradation of curcumin [12]. Vanillin, vanillic acid, ferulic aldehyde and ferulic acid have been identified as the degradation products of curcumin [29]. In general, the curcumin impregnation in alkaline medium was efficient indicating that chitosan is a potential option for this application.

The encapsulation efficiency of the dye in the microspheres was evaluated through the relation between the amount of dye present in the microspheres at pH 4.0 and the respective theoretical value.

The values for the encapsulation efficiency were: 44.80% ± 1.16; 66.14% ±1.15 and 86.64% ± 2.44 for the microspheres at pH 9.0, 9.5 and 10.0, respectively. These data are consistent with the data obtained in the capillary electrophoresis analysis where a greater concentration of curcumin was verified in the sample at pH 10.0 (Fig. 3A).

The impregnated sample at pH 10.0 was characterized by Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), Optical Microscopy and Differential Scanning Calorimetry (DSC).

Figure 4 shows the infrared spectra of chitosan and dye-loaded microspheres. In the IR spectrum for the chitosan (Fig. 4a), characteristic absorption bands were displayed at 3442 cm$^{-1}$ relating to –OH groups, and two other bands located at 2925 and 2860 cm$^{-1}$ are typical of C–H

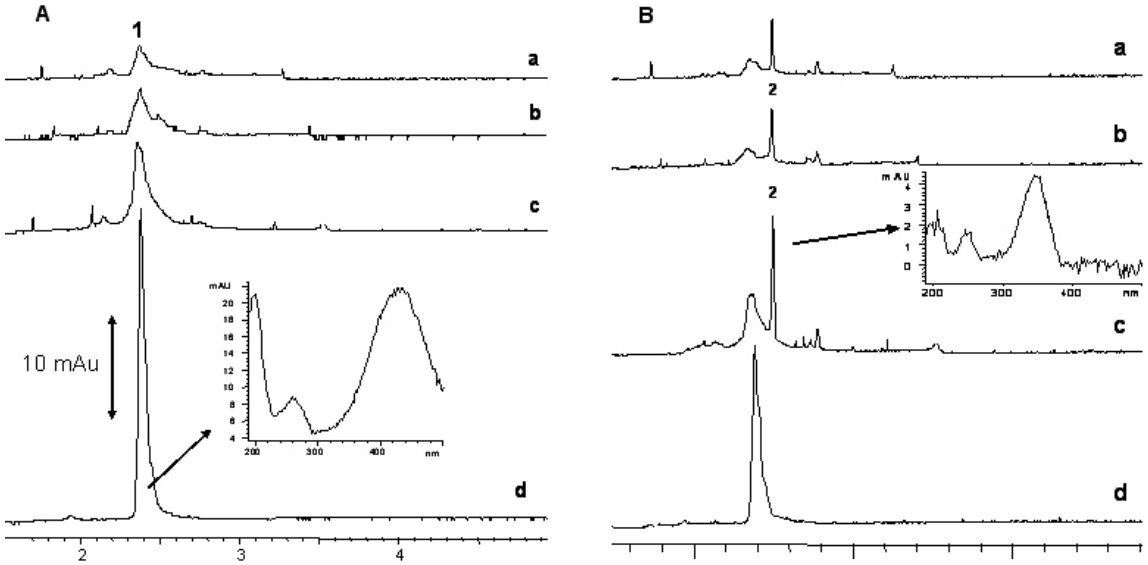


**Figure 3**. Electrophoretogram of impregnated microespheres at pH 9.0 (a), pH 9.5 (b), pH 10.0 (c) and pure standard curcumin (d) at 429 nm (**A**) identifying curcumin (1) and 360 nm (**B**) identifying vanilin (2) in the impregnated microespheres.

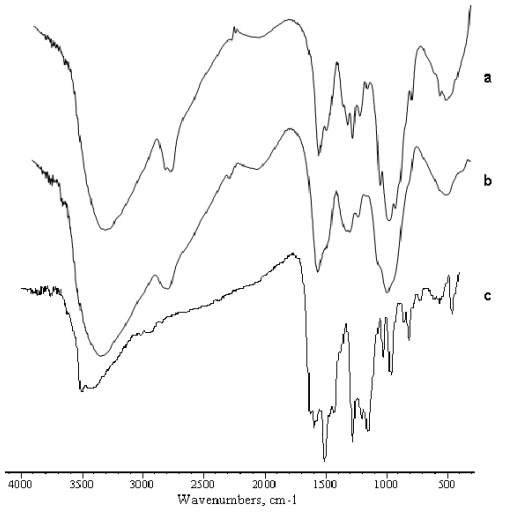


**Figure 4**. FTIR spectra of chitosan microspheres (**a**) impregnated chitosan microspheres (**b**) and dye (**c**).

groups. Over the range of 1500–1700 $cm^{-1}$ both amide and amine bands are observed. The amide I band, a C-O stretching mode together with an N-H deformation mode, is located at 1650 $cm^{-1}$ [30-32]. The band at 1567 $cm^{-1}$ is assigned to the $NH_2$ groups of the chitosan (Fig. 4a). The FT-IR spectrum of the dye (Fig. 4c) shows characteristic absorption bands at 1620 $cm^{-1}$ assigned to the C=O group of ketone; 1562 and 1420 $cm^{-1}$ to C=C stretching of the aromatic ring and 1070 $cm^{-1}$ to stretching of the C–O–C of the ester [33]. It was observed that the peak at 1567 $cm^{-1}$ relating to the $NH_2$ group of the chitosan was shifted in the case of the impregnated sample to 1576 $cm^{-1}$ (Fig. 4b). The FTIR results suggested an interaction between chitosan and dye in the impregnated microspheres.

The SEM analysis performed on all batches of microspheres showed that they are spherical with smooth surfaces. It also indicated the non-porous and crack-free nature of the microspheres (Fig. 5a), indicating the formation of a continuous film on the wall. The mean particle size of the loaded chitosan microspheres was 796 (± 20) µm, whereas for non-loaded chitosan microspheres it was 776 (± 25) µm. As is evident from Fig. 5b, the inside of the chitosan microspheres also had a non-porous and crack-free structure. The analysis of the micrographs of the loaded microsphere surfaces and cross-sections, together with the visually observed change in the microsphere surface coloration to an intense chestnut-yellow, indicate a high efficiency of impregnation.

The optical microscopy analysis showed that the dye moved weakly through the microsphere without reaching its nucleus (Fig. 6). A greater dye concentration gradient was observed at the surface of the microsphere in the direction toward the center (diffuse layer) (Fig. 6b).

Figure 7 shows the results obtained from the DSC analysis of the chitosan microspheres, the dye and the dye-loaded microspheres. Scanning calorimetry (DSC) was used to confirm the possible interaction between the polymeric matrix and the dye. The chitosan microspheres (Fig. 7a) show a large endothermic peak at around 80-120 °C which is due to the evaporation of the water present in the sample, followed by a sharp exothermic peak at around 299°C corresponding to the degradation temperature of the polymer [25,34]. An endothermic peak was observed at 172 °C corresponding to the curcumin dye melting point [21] (Fig. 7c). The DSC profile of the dye-loaded microspheres (Fig. 7b) shows two exothermic peaks, the first at 284 °C and the second at around 303 °C, which may be attributed to the degradation temperatures of the dye and polymer, respectively. The DSC analysis indicated the presence of dye in the impregnated microspheres and it confirmed a physical interaction between the dye and the polymer in the impregnated microspheres.

The release of dye from the impregnated chitosan microspheres was monitored, as a function of time, under different buffer solution conditions. Figure 8 shows a plot of the fraction of

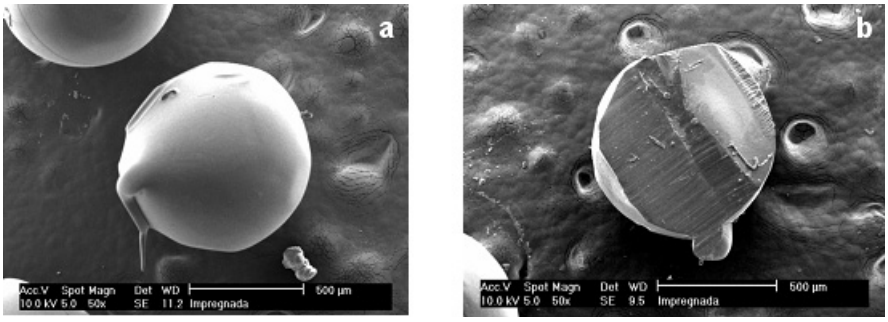


**Figure 5**. Scanning electron micrographs of chitosan microspheres impregnated with dye (**a**) and cross-section of impregnated chitosan microsphere (**b**).

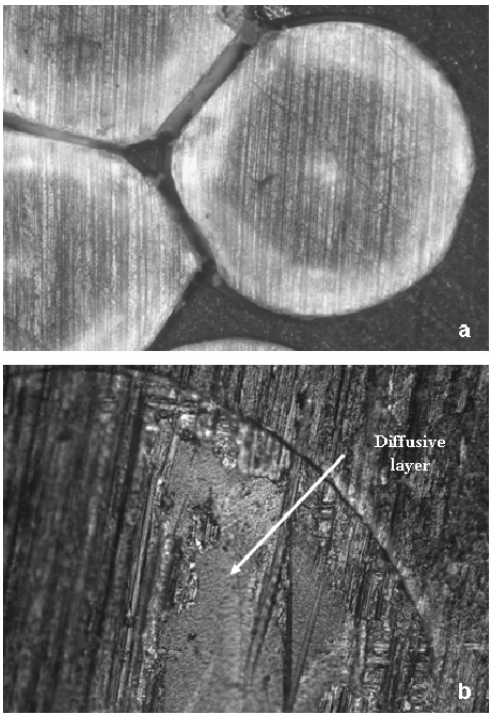


**Figure 6**. Optical Micrographs of chitosan microspheres (**a**) and impregnated chitosan microspheres (**b**).

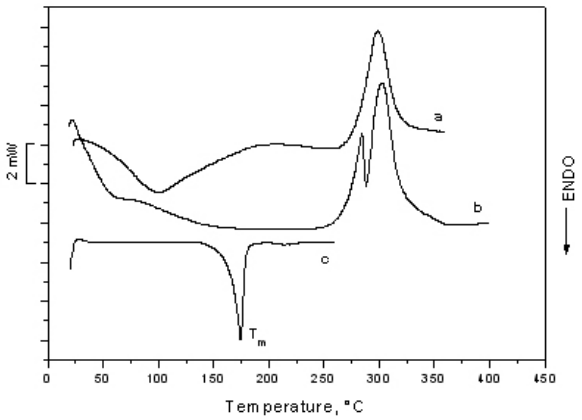


**Figure 7**. DSC results for chitosan microspheres (**a**), impregnated chitosan microspheres (**b**) and dye (**c**).

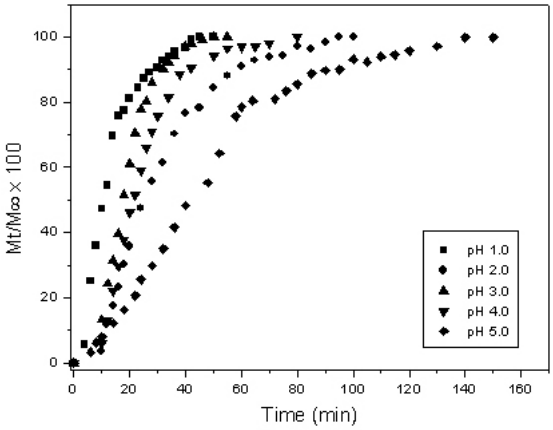


**Figure 8**. Release profiles of curcuma dye-impregnated microspheres at different pH values at 25 °C.

dye released as a function of time, where $M_t$ is the amount of dye released at time t and $M_\infty$ is the amount of dye released as time approaches infinity. The release of dye at pH 1.0, 2.0, 3.0 and 4.0 began after 10 min. The corresponding time for pH 5.0 was 20 min, due to the time required for the permeation of the dye to reach the medium from the inner cavities of the microsphere. The time required for a 50% dye release was with 10-42 minutes in the pH range of 1-5 (Table 1). A faster release was observed at pH 1.0. It can be seen from Fig. 8 that the dye was released from the chitosan in less than 3 h, regardless of the pH, although most of the microspheres dissolved within 1 h.

In recent years, many attempts have been made to elucidate the mechanism of solute release from hydrophilic matrices [31–38]. It has been shown that solute release from hydrophilic matrices involves a complex interaction between swelling, diffusion, erosion and polymeric chain relaxation [35].

| | Swelling | | | Equation 3 | |
|---|---|---|---|---|---|
| **pH** | **Time** [a] | **Swelling %** | **Time required for 50% release** [b] | **n** | **$R^2$** |
| 1.0 | 8 | 32.18 | 10 | 1.13 | 0.9959 |
| 2.0 | 24 | 36.56 | 23 | 1.68 | 0.9918 |
| 3.0 | 14 | 82.30 | 19 | 1.85 | 0.9977 |
| 4.0 | 20 | 71.20 | 21 | 1.69 | 0.9946 |
| 5.0 | 28 | 32.18 | 42 | 1.33 | 0.9970 |

**Table 1**. Parameters of swelling and release from chitosan matrix containing the natural dye curcuma. [a] time required to obtain microspheres of constant mass, min. [b] min.

Equation [**3**] is currently used for the analysis of solute release processes in order to gain an understanding of the predominant mechanisms [39]:

$$\frac{M_t}{M_\infty} = k \cdot t^n \qquad [3]$$

where $M_t/M_\infty$ is the proportion of dye released at time $t$, $k$ is a kinetic constant and n is the release exponent, indicative of the mechanism of solute release [36,38,40]. Equation [**3**] is valid only for the early stages (≤ 60%) of solute release.

The values for the release parameter, n, were determined from the slope of the plot of log ($M_t/M_\infty$) versus log $t$. The results are summarized in Table 1. The values obtained for the diffusional exponent are n > 1.0 regardless of the pH and reveal a Super Case II transport release mechanism.

The swelling capacity of the impregnated microspheres is shown in Table 1. The results indicate that the swelling behavior was affected by the pH, suggesting that the amino groups of chitosan are ionized in acidic medium (pKa chitosan = 6.5) [41] and this causes hydration and formation of a hydrogel matrix through which the dye has to pass by dissolution and diffusion [34,35].

In the Super Case II transport release mechanism the polymeric chains must first be arranged (swelling and relaxation) to allow the diffusion process [36,37,42]. In this regard, the chain mobility is fundamental to the solute transfer kinetics [37,43]. This type of transport has also been reported by other researchers [35,41,45].

## CONCLUSIONS

Curcumin has many applications in the food and dye industries and in agriculture. In this study, chitosan was shown to be a polymer suitable for the encapsulation of the natural dye curcuma. Under the conditions of this study, the biopolymer chitosan increased the dye solubility in acidic medium through an alternative method that could be applied to pharmaceutical and food formulations.

## REFERENCES


1. Downham, A. & P. Collins (2005) *Int. J. Food Sci. Technol.* **35**: 5-22.
2. Kiokias, S. & M. H. Gordon (2003) *Food Chem.* **83**: 523-9.
3. Mehta, K., P. Pantazis, T. Mcqueen & B. B. Aggarwal (1997) *Anticancer Drugs* **8**: 470-81.
4. Masuda, T., K. Hidaka, A. Shinohara, T. Maekawa, Y. Takada & H. Yamaguchi (1999) *J. Agric. Food Chem.* **47**: 71-7.
5. Motterlini, R., R. Foresti, R. Bassi & C. J. Green (2000) *Free Radic. Biol. Med.* **28**: 1303-12.
6. Sharma, R.A., A.J. Gescher & W.P. Steward (2005) *Eur. J. Cancer* **41**: 1955-68.
7. Aggarwal, B.B., A. Kumar & A.C. Bharti (2003) *Anticancer Res.* **23**: 363-98.
8. Joe, B., M. Vijaykumar & B.R. Lokesh (2004) *Crit. Rev. Food. Sci. Nutr.* **44**: 97-111.
9. Duvoix, A., R. Blasius, S. Delhalle, M. Schnekenburger, F. Morceau, E. Henry, M. Dicato & M. Diederich (2005) *Cancer Lett.* **223**: 181-90.
10. Gopinath, D., M.R. Ahmed, K. Gomathi, K. Chitra, P.K. Sehgal & R. Jayakumar (2004) *Biomaterials* **25**: 1911-7.
11. Martin, A. (1996) *Dermatol. Surg.* **22**: 156-60.
12. Price, L.C. & R.W. Buescher (1997) *J. Food Sci.* **62**: 267-9.
13. Tonnesen, H.H. & J. Karlsen (1985) *Z. Lebensm. Unters. Forsch.* **180**: 402-4.
14. Souza, C.R.A., S.F. Osme & M.B.A. Gloria (1997) *J. Food Process. Pres.* **21**: 353-6.
15. Hergenhahn, M., B. Bertram, M. Wiessler & B.L. Sorg (2003) *U.S. Pat. Appl.* 2003/0153512 A1.
16. Todd, P.H. (1991) *Patentschrift DE* 40 26 118 C2.
17. Wang, Y.J., M.H. Pan, A.L. Cheng, L.I. Lin, Y.S. Ho, C.Y. Hsieh & J.K. Lin (1997) *J. Pharm. Biomed. Anal.* **15**: 1867-76.
18. Wang, F., J.H. Yang, X. Wu & S.F. Liu (2005) *Spectrochim. Acta A-M.* **61**: 2650-6.
19. Herek, L.C.S., R. C. Oliveira, A. F. Rubira & N. Pinheiro (2006) *Braz. J. Chem. Eng.* **23**: 227-34.
20. Prachayawarakorn, J. & W. Kryratsamee (2006) *J. Appl. Polym. Sci.* **100**: 1169-75.
21. Vidyalakshmi, K., K.N. Rashmi, T.M.P. Kumar & M.P. Siddaramaiah (2004) *J. Macromol. Sci. A* **41**: 1115-22.
22. Kittur, F.S. & R.N. Tharanathan (2003) *Crit. Rev. Food Sci. Nutr.* **43**: 61-87.
23. Kumar, M.N.V.R. (2000) *React. Funct. Polym.* **46**: 1-27.
24. Higuera-Ciapara, I., l.F. Valenzuela, F.M. Goycoolea & W.A. Monal (2004) *Carbohydr. Polym.* **56**: 41-5.
25. Souza, T.C.R., A.L. Parize, I.M.C. Brighente, V.T. Fávere & M.C.M. Laranjeira (2005) *J. Microencapsul.* **22**: 511-20.

26. Yuan, K., Q. Weng, H. Zhang, J. Xiong & G. Xu (2005) *J. Pharm. Biomed. Anal.* **38**: 133-8.
27. Bernabé-Pineda, M., M.T. Ramírez-Silva, M. Romero-Rombo, E.G. Vergana & A.R. Hernández (2004) *Spectrochim. Acta A* **60**: 1091-7.
28. Tonnesen, H.H. (2006) *Pharmazie* **61**: 696-700.
29. Tonnesen, H.H., J. Karlsen & G.B. Henegouwen (1986) *Z. Lebensm. Unters. Forsch.* **180**: 116-22.
30. Domszy, J.G. & G.A.F. Roberts (1985) *Makromol. Chem.* **186**: 1671-7.
31. Jackson, M., P.I. Harris & D.J. Chapman (1989) *Mol. Struct.* **214**: 329-55.
32. Le Gal, J M., M. Manfait & T.J. Theophanides (1991) *Mol. Struct.* **242**: 397-407.
33. Pan, C.H.J., J.J. Tang, Y.J. Weng, J. Wang & N. Huang (2006) *J. Control. Release* **116**: 42-9.
34. Hanafi, M.M., S.M. Eltaib & M.B. Ahmad (2000) *Eur. Polym. J.* **36**: 2081-8.
35. Harland, R.S., A. Gazzaniga, M.E. Sangalli, P. Colombo & N.A. Peppas (1988) *Pharm. Res.* **5**: 488-94.
36. Ferrero, C., A. Muñoz-Ruiz & M.R. Jiménez-Castellano (2000) *Int. J. Pharm.* **202**: 21-8.
37. Roy, D.S. & B.D. Rohera (2002) *Eur. J. Pharm. Sci.* **16**: 193-9.
38. Llabot, J.M., R.H. Manzo & D.A. Allemandi (2004) *Int. J. Pharm* **276**: 59-66.
39. Popa, M.I., N. Aelenei, V.I. Popa & D. Andrei (2000) *React. Funct. Polym.* **45**: 35-43.
40. Korsmeyer, R.W., R. Gurny, E. Doelker, P. Buri & N.A. Peppas (1983) *Int. J. Pharm.* **15**: 25-35.
41. Ritger, P.L. & N.A. Peppas (1987) *J. Control. Release* **5**: 23-36.
42. Siepmann, J. & N.A. Peppas (2001) *Adv. Drug Deliv. Rev.* **48**: 139-57.
43. Ranga Rao, K.V., P. Devi & P. Buri (1988) *Drug Dev. Ind. Pharm.* **14**: 2299-320.
44. Munday, D.L. & P.L. Cox (2000) *Int. J. Pharm.* **203**: 179-92.
45. Osifo, P.O., A. Webster, H. van der Merwe, H.W.J.P. Neomagus, M.A. van der Gun & D.M. Grant (2008) *Bioresource Technol.* **99**: 7377-82.