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Ionically Cross-linked Chitosan/Tripolyphosphate Microparticles for the Controlled Delivery of Pyrimethamine

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Published: 01 May 2011
Ibnosina J Med BS 2011,3(3):77-88
Received: 24 July 2010
Accepted: 14 January 2011
This article is available from: http://www.ijmbs.org

Abstract
Chitosan ionically cross-linked with tripolyphosphate at regulated temperatures (25°C, 40°C, and 50°C) and varying cross-linking times (30 min, 2 h and 4 h respectively) was used to form microparticles employed in the encapsulation of pyrimethamine, an antiprotozoal drug. The yields, equilibrium moisture contents, percentage concentration, swelling characteristics, entrapment efficiency, release properties, infrared spectroscopy, and differential scanning calorimetry of the formulated microparticles were evaluated. The yield of microparticles produced ranged from 0.3515 to 0.7749 g per 100 ml of cross linking solution. The products possessed relatively little amounts of moisture (0.22 – 3.04 % w/v). The entrapment efficiencies ranged from 25.55 to 99 % with the product formed at ambient temperature and cross linking time of 30 min possessing the highest efficiency. The swelling kinetics on the microcapsules revealed that all the products swelled in the various pH media following mainly anomalous sorption mechanism with a few diffusion controlled mechanism. The greatest swellings however occurred at the swelling medium of pH 1 while the least swelling occurred at pH 7. Spectral and differential scanning calorimetric properties of the chitosan used in the study were consistent with those of standard chitosan. The infrared spectroscopy and differential scanning calorimetry of the products confirmed that encapsulation actually occurred with the spectral characters of the products differing from those of the parent constituents (chitosan, tripolyphosphate and pyrimethamine). Based on these factors, tripolyphosphate cross-linked chitosan microparticles present a suitable matrix for the controlled release of pyrimethamine.

Keywords: Pyrimethamine, antiprotozoal, microparticles, spectral, chitosan, controlled delivery, spectral properties.

Introduction

Pyrimethamine, {5-(4-chlorophenyl)-6-ethyl-2, 4- pyrimi-

dinediamine}, is widely employed in the treatment of protozoal infections. It is specifically used in the treatment and prevention of malaria and in the treatment of toxoplasma infections in immunocompromised patients (1-3). It interferes with folic acid synthesis through inhibition of the enzyme dihydrofolate reductase (4). It has numerous side effects particularly when used with a sulphonamide (5). Search for a sustained release form of this important antiprotozoal agent was necessitated by the need firstly to improve compliance by patients on pyrimethamine, thereby minimizing the development of microbial resistance and secondly to reduce the frequency of administration thus contributing to minimizing the extensive side effects associated with the drug. Various techniques including microencapsulation have been employed in the design of controlled release formulations (6-10). Chitosan / tripolyphosphate complex beads (microparticles) have been tried for controlled release of model drugs (11). Chitosan is deacylated chitin and has excellent biodegradable and biocompatible characteristics, with unique polymeric cationic character, as well as gel and film forming properties. Consequently, it has been examined extensively in the pharmaceutical industry for its potential in the development of drug delivery systems (12-16). Chitosan has been widely researched for biomedical applications such as wound healing, drug delivery systems, coatings and tissue engineering, as well as applications in food, cosmetics and agricultural industries (17-21). Chitosan has been gaining increasing importance in the pharmaceutical field owing to its good biocompatibility, low toxicity, and biodegradability (22,23). The degradation products of chitosan are nontoxic, nonimmunogenic, and noncarcinogenic. Chitosan microparticles cross-linked with glutaraldehyde were shown to be long-acting biodegradable carriers suitable for use in microparticles delivery system (24-27). In order to surmount the toxicity problems (15, 28, 29) associated with chemical cross linking such as with glutaraldehyde, ionic cross linking has been utilized in the production of chitosan/tripolyphosphate microparticles. In this study, pyrimethamine-loaded microparticles from chitosan/TPP were developed and evaluated for the possible controlled release delivery of pyrimethamine.

Materials and Methods

Materials

Analytical grades of dimethyl sulphoxide (Riedel De Haen, Hannover), ethanol (Tedia, USA), pyrimethamine (Sigma, USA), 1 % acetic acid (Laboratory stock) and sodium tripolyphosphate, $Na_5P_3O_{10}$ (Sigma Aldrich, USA) were used in the study. Chitosan was produced in the Hydrosoluble Laboratory of the Institute of Macromolecules, Federal University of Rio de Janeiro, Brazil, by a heterogeneous deacetylation of chitin obtained from shrimp, Penaeus schmitti.

Preparation of chitosan/tripolyphosphate microparticles

A modification of previously established procedure was employed (6). Briefly, a 250 mg quantity of chitosan was added in bits with constant stirring to 50 ml of 1 % acetic acid until complete dissolution was obtained. A 5 mg quantity of pyrimethamine powder was introduced into the chitosan solution with constant stirring at ambient temperature, until completely dissolved. The resulting solution was then introduced in droplets using a peristaltic pumping device (Industria Brasiliera, Brazil) into 50 ml of an 8% w/v solution of sodium tripolyphosphate with constant stirring at ambient temperature. The mixture was allowed to stir undisturbed for further 30 min. Thereafter, the mixture was centrifuged for 15 min at 18^{O}C at a speed of 4000 rpm. The supernatant was discarded and the microparticles washed severally with water, are dried in an oven at 50^{O}C for 24 h. A second batch of microparticles was prepared without the drug, for the purpose of comparison. The capsules were then pulverized in a mortar and stored in the desicator for further use.

Effect of stirring time and stirring temperature on microencapsulation

The microcapsules were prepared according to the earlier outlined procedures above. However, instead of stirring for 30 minutes, the mixture was stirred for 2 hours. Another batch was prepared and stirred for 4 hours. The same procedure for preparing the microcapsules was also followed, but the addition of the chitosan into tripolyphosphate was effected at a temperature of 40^{O}C in a thermostated water bath. Another batch was produced at a temperature of 50^{O}C.

Moisture content of prepared microcapsules and swelling studies on them

A 10 mg quantity of each of the prepared microcapsules was placed in a pre-heated and pre-weighed crucible. The crucible containing the microcapsules was placed in an oven at 105^{O}C for 3 h. This is then cooled and re-weighed. The heating, cooling, and weighing process was continued at hourly intervals until constant weights were obtained. The difference in weight, expressed as a percentage of the original weight of microcapsules represents the moisture content of the microcapsule. Three replicated

measurements were taken. The water sorption capacity of the prepared microcapsules was determined by swelling given quantities of dry microcapsules in de-ionized water at ambient temperature (25^{O}C) for at least 24 h. The wet weight of the swollen microcapsules was determined by first blotting the microcapsules with filter paper to remove surface water and then weighing immediately. The percentage swelling of the pyrimethamine microcapsules was calculated from Equation 1 (Appendix). The swelling behavior of the pyrimethamine microcapsules in media of various pH (1,7,14) were determined. The swelling ratio was obtained by weighing the initial and swollen samples at various time intervals. The amount of water absorbed was reported as a function of time.

Drug entrapment efficiency

The entrapment efficiency of the pyrimethamine microcapsules was determined by dissolving 1.5 mg of the microcapsules in 20 ml of ethanol. This was achieved by stirring the solution using a magnetic stirring device for 3 hours.

Table 1: Entrapment efficiencies (%) and Equilibrium moisture content (%) of the Different Pyrimethamine microcapsules.

Different Pyrimethamine Microcapsules	Entrapment Efficiency (%)		Equilibrium Moisture Content (%)
	Batch 1	Batch 2	
Chitosan/TPP + Pyrimethamine at ambient temperature and 30 mins cross linking time	93.46	99.00	0.669
Chitosan/TPP + Pyrimethamine at ambient temperature and 2 hr cross linking time	76.22	94.76	1.460
Chitosan/TPP + Pyrimethamine at ambient temperature and 4 hr cross linking time	25.55	99.00	0.603
Chitosan/TPP + Pyrimethamine at 40^{O}C and 30 mins cross linking time	59.33	99.00	1.484
Chitosan/TPP + Pyrimethamine at 40^{O}C and 2 hr cross linking time	42.96	85.90	0.858
Chitosan/TPP + Pyrimethamine at 40^{O}C and 4 hr cross linking time	72.66	98.60	1.285
Chitosan/TPP + Pyrimethamine at 50^{O}C and 2 hr cross linking time	25.95	95.30	0.220
Chitosan/TPP without Pyrimethamine at ambient temperature and 30 mins cross linking time	-	-	0.705
Chitosan/TPP without Pyrimethamine at ambient temperature and 2 hr cross linking time	-	-	2.030
Chitosan/TPP without Pyrimethamine at ambient temperature and 4 hr cross linking time	-	-	3.030
Chitosan/TPP without Pyrimethamine at 40^{O}C and 2 hr cross linking time	-	-	2.900
Chitosan/TPP without Pyrimethamine at 50^{O}C and 2 hr cross linking time	-	-	3.040

Table 2: Model equation 2 swelling constants K, and n for pure chitosan and the various microcapsules in different swelling media

Sample	pH/Swelling Medium	K	N	R
WPM 30 Amb	1	0.0697	0.39	0.9656
	7	0.8426	-0.03	0.2102
	14	0.8447	-0.02	0.0007
PM 30 Amb	1	0.0614	0.42	0.8708
	7	0.7499	-0.04	0.2669
	14	0.7640	-0.03	0.1797
WPM 2H Amb	1	0.1188	0.38	0.8952
	7	0.7386	-0.06	0.6322
	14	0.8478	-0.02	0.1016
PM 2H Amb	1	0.2107	0.31	0.4454
	7	0.8285	0.05	0.2409
	14	0.8177	0.05	0.2256
WPM 4H Amb	1	0.2452	0.37	0.9302
	7	0.9171	0.04	0.2086
	14	0.6904	0.08	0.3672
PM 4H Amb	1	0.7741	0.08	0.3328
	7	0.7401	0.05	0.4353
	14	0.9489	0.05	0.2472
PM 30 40	1	0.0338	0.62	0.9834
	7	0.9842	0.02	0.0719
	14	0.7338	0.09	0.4945
WPM 2H 40	1	0.1138	0.37	0.8484
	7	0.7586	0.08	0.4120
	14	0.7360	0.04	0.6680
PM 2H 40	1	0.1025	0.33	0.9765
	7	0.9365	0.01	0.0188
	14	0.8337	0.07	0.3757
PM 4H 40	1	0.2904	0.20	0.7915
	7	0.6587	-0.11	0.4619
	14	0.5265	0.10	0.9083
PM 30 50	1	0.0728	0.50	0.9913
	7	0.8794	0.03	0.0939
	14	0.5204	0.16	0.5669
WPM 2H 50	1	0.0346	0.58	0.9044
	7	0.7870	0.05	0.1716
	14	0.5703	0.13	0.5582
PM 2H 50	1	0.1161	0.42	0.6807
	7	0.8188	0.04	0.1824
	14	0.4424	0.21	0.4237
PM 4H 50	1	0.0902	0.46	0.8970
	7	0.5585	-0.12	0.8948
	14	0.9234	-0.01	0.0987

The amount of drug loaded was determined by measuring the absorbance of the solutions using the UV-Vis spectrophotometer at 200.99 nm wavelength and reference to the standard Beer´s curve using equation 2 (Appendix).

In vitro release study

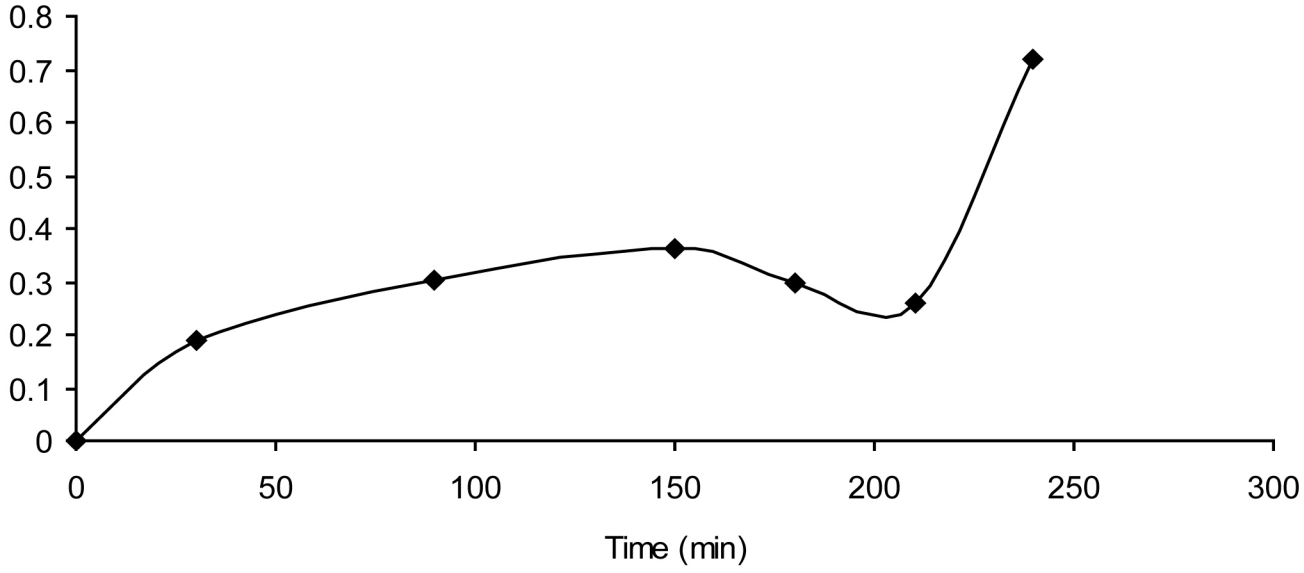

Figure 1. In vitro release profile in simulated gastric juice microcapsule prepared at ambient temperature and 30 min cross-linking time.

To predict the possible in vivo release pattern of the drug, a 5 mg quantity of the microcapsules was introduced into 100 ml of 0.1 M hydrochloric acid solution, stirred continuously at a speed of 50 rpm and maintained at a temperature of 37 ^{O}C. At regular time intervals, 1 ml aliquots of the solution were withdrawn and diluted with fresh 0.1 M HCl. The amount of pyrimethamine released was determined by measuring the absorbance in a UV-Vis spectrophotometer (200.99 nm) using 0.1 M HCl as blank and reference to the standard Beer´s plot. After each withdrawal, an equivalent volume of fresh 0.1 M HCl was re-introduced into the medium to maintain the volume constant.

Infrared spectroscopy

The various samples were characterized by the Fourier transform infrared spectrometry, recorded on KBr pellets. All spectra were obtained using a Perkin-Elmer model 1700 spectrophotometer (Massachusetts, USA), from 4000 to 400 cm^{-1} at data acquisition rate of 2 cm^{-1} per point. At least 20 scans were done. Duplicate samples of CS, and possible microparticles were analyzed and spectra for the duplicate runs averaged.

Differential scanning calorimetry

Thermograms were obtained for the chitosan, Pyrimethamine and cross linked microparticles using TA instruments calorimeter (model Q-1000, New Castle, USA). Samples (3 mg) were crimped in a standard aluminium pan and heated from 25 to 350ºC at a heating constant rate of 10ºC min^{-1} under constant purging of nitrogen.

Results

Entrapment efficiency and equilibrium moisture contents:

The microcapsules generally had quite good entrapment efficiencies with all the products showing average entrapment efficiency greater than 50% (Table 1). The highest average efficiency of over 96% was recorded by microcapsules formulated at ambient temperature and a cross linking time of 30 minutes. The lowest entrapment efficiency was recorded by formulation at ambient temperature and 4 h cross linking time. The equilibrium moisture contents of the Pyrimethamine microcapsules were minimal (Table 1). The greatest moisture content (3.040%) was observed in the sample without the drug, produced at 50^{O}C and 2 h cross linking time whilst the sample produced at ambient temperature and 4 h cross linking time, had the least moisture content (0.603.%.)

Swelling characteristics and release studies

The swelling kinetics of the pyrimethamine microcapsules are presented in Table 2. These show that all the microcapsules swell upon contact with the various swelling media, and the degree of swelling was shown to be pH-dependent. The result of the preliminary release study is

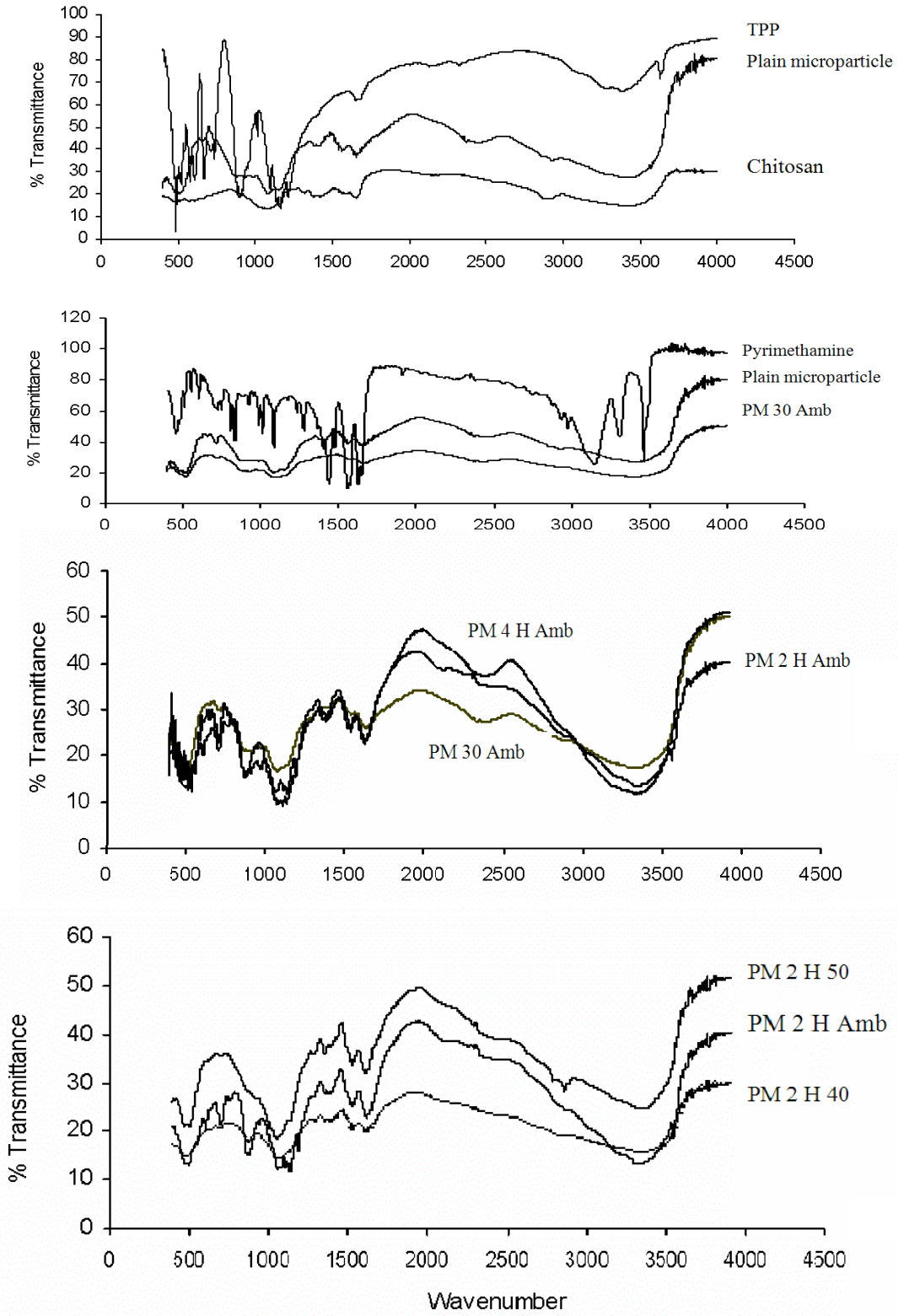

Figure 2. Fourier-transform infra red spectrals of pure chitosan, TPP and plain microparticles prepared without pyrimethamine (Panel A, top.), of pyrimethamine, plain microparticles prepared at ambient temperature and 30min cross-linking time (PM 30 Amb) (Panel B), of pyrimethamine microparticles prepared at ambient temperature and 30min cross-linking time (PM 30 min), 2 hours cross-linking time (PM 2 H Amb) and 4 hours cross-linking time (PM 4 H Amb) and finally of pyrimethamine microparticles prepared at different temperatures – ambient (PM 30 Amb), 40oC (PM 2 H 40), 50oC 0 (PM 2 H 50) and at 2 h cross-linking time (Panel D, lowest). X-axis shows wave number and Y-axis shows percentage transmittance.

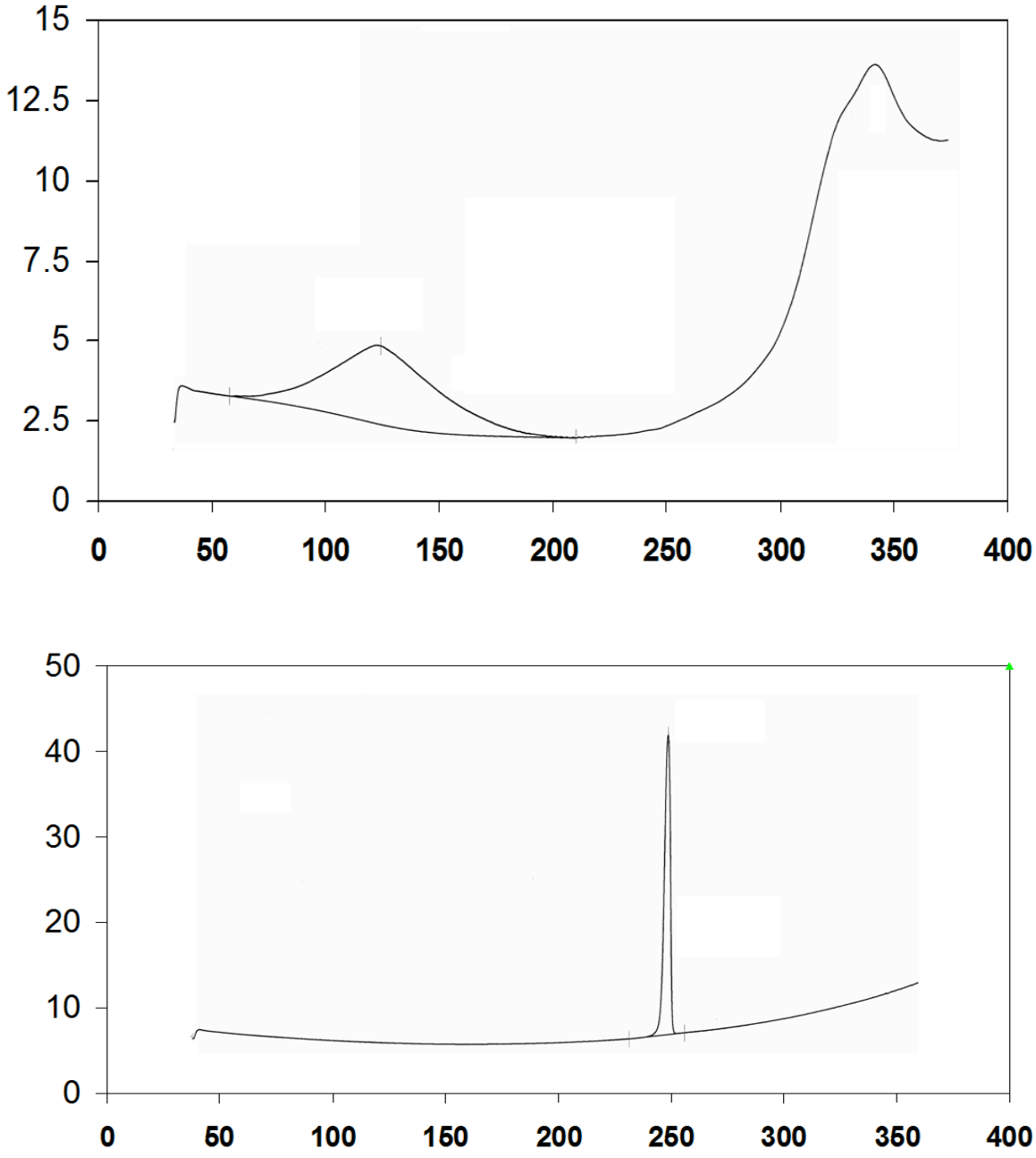

Figure 3. Differential scanning calorimetry curve of plain Chitosan (Panel A, Upper) and plain Pyrimethamine (Panel B; Lower). The X-axis represents the temperature whereas the Y axis represents heat flow.

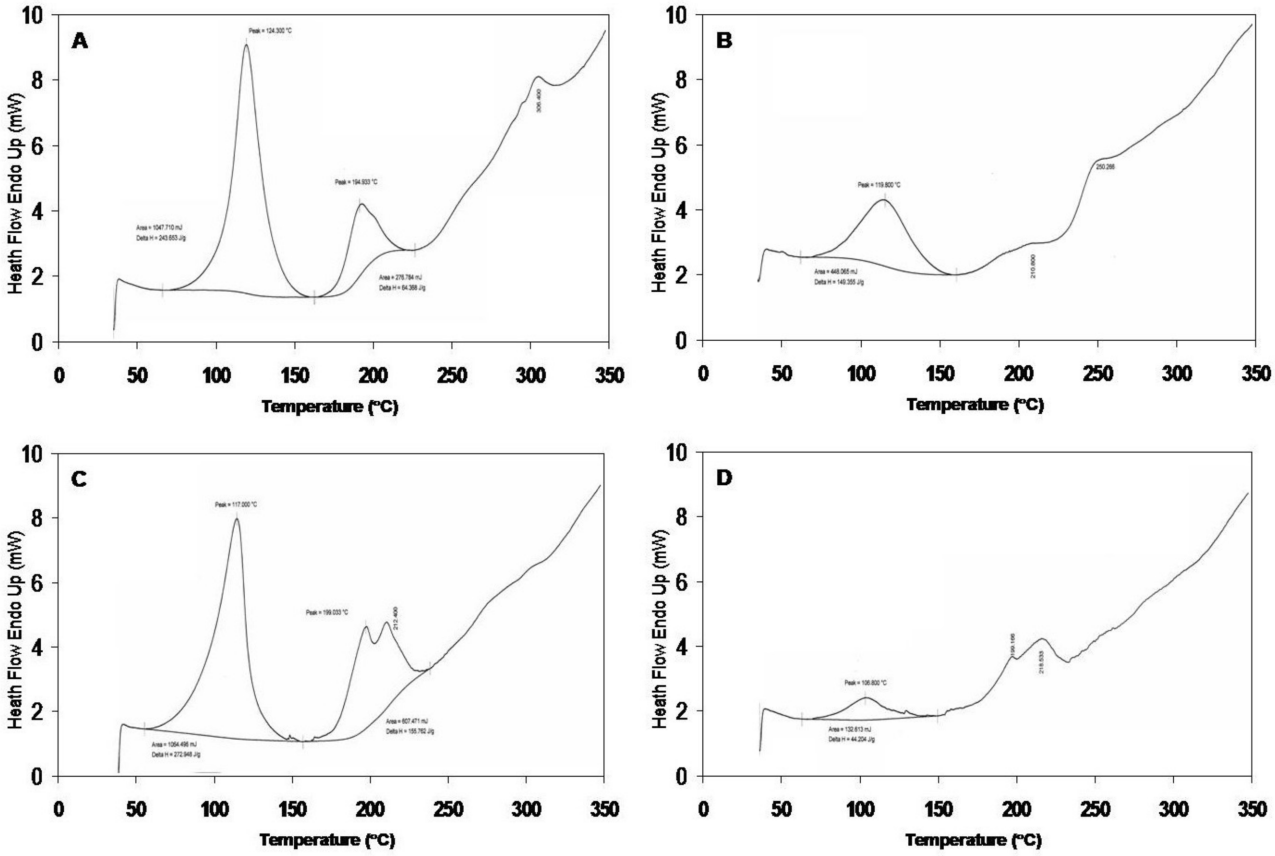

Figure 4. Differential scanning calorimetry curves of A. Pyrimethamine-loaded microparticles prepared at ambient temperature and 4 hours cross-linking time, B. Pyrimethamine-loaded microparticles prepared at 50oC and 2 hours cross-linking time, C. No pyrimethamine-loaded microparticles and D. Pyrimethamine-loaded microparticles prepared at ambient temperature and 30 minutes cross-linking time.

presented in Figure 1. The formulation seemed to exhibit prolonged release properties as the microcapsules released less than 50 percent of their drug content within 24 hours of the study.

Infrared spectroscopy

The Fourier-transform infrared spectroscopy (FTIR) of chitosan, TPP, pyrimethamine plain chitosan / TPP complexes and pyrimethamine-loaded microparticles are presented in Figure 2. It shows that the microparticle exhibited a combination of the typical absorption bands of panel B TPP and chitosan. The microparticles resulted from the interaction between the positively charged amino group of chitosan and the negatively charged counter ion of TPP. The absorption band at 3510 cm^{-1} attributed to –NH group in chitosan was broadened by the physical interactions with TPP, while a shoulder appeared at 1655 cm^{-1} due to chitosan amide at same position after cross linking with TPP, and indicates interactions of chitosan amide with added polyions. The observed absorption bands at 1278 and 1103 cm^{-1} have been assigned to –P=O groups of polyphosphate anion. In the cross linked microparticles, the $–NH_2$ bending vibration was observed at 1630 cm^{-1} in place of 1590 cm^{-1} due to the interactions of TPP ions with $–NH_3^+$ ions of chitosan. The spectrum of the drug-loaded microparticles is dominated by the chitosan / TPP complex bands. The microparticles prepared at different cross linking times exhibited similar absorption bands but with slight differences at band regions 960, 1100 and 1600 cm^{-1} (Figure 2) Thus, confirming the occurrence of encapsulation with slightly varying degrees of cross-linking. Also, it could be reflected from the Figure given the clear deepening of absorption intensities at 3510 cm^{-1} that higher cross-linking times above 30 min generally resulted in higher degrees of cross-linking between the chitosan and TPP, and this trend can possibly limit the drug entrapment

efficiency. Increasing the temperature of cross-linking led to a slight change in the absorption characteristics of the microparticles at regions 880 and 2800 cm^{-1} (Figure 2) with generalized changes in the absorption intensity at 1650 cm^{-1} which decreased correspondingly, thus suggesting that the temperature-modulated degree of cross-linking was highest for microparticles prepared at 25°C (ambient), followed by 40°C and then 50°C.

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry curves of Pyrimethamine-loaded microparticles prepared at ambient temperature and 4 hours cross-linking time, Pyrimethamine-loaded microparticles prepared at 50°C and 2 hours cross-linking time, no pyrimethamine-loaded microparticles and Pyrimethamine-loaded microparticles prepared at ambient temperature and 30 minutes cross-linking time are shown in Figure 4. The thermal behaviours of the drug loaded microparticles was compared to those of the original species (pyrimethamine, chitosan, TPP and the plain unloaded microparticles), differential scanning calorimetry measurements presented in Figure 3 and 4. The DSC curve of pyrimethamine (Figure 3) shows a sharp endothermic peak at 242 ^{O}C typical of the drug (37).

The DSC curve for the plain chitosan / TPP microparticles (Figure 4) exhibits two broad endothermic events in the ranges 75 – 140 ^{O}C and 190 – 240 ^{O}C, the first assigned to loss of water and the second one to thermal decomposition (38), as the compound starts to lose mass in this range. The DSC curve of the microparticle shows an apparent combination of events of chitosan, TPP and pyrimethamine.

Discussion

This study attempted to develop and evaluate ionically cross-linked with tripolyphosphate at regulated temperatures (25°C, 40°C, and 50°C) and varying cross-linking times (30 min, 2 h and 4 h respectively) was used to form microparticles employed in the encapsulation of pyrimethamine, a commonly used antiprotozoal drug. The yields, equilibrium moisture contents, percentage concentration, swelling characteristics, entrapment efficiency, release properties, infrared spectroscopy and differential scanning calorimetry of the formulated microparticles were evaluated.

The low loading efficiency recorded may be indicative of reduced loss of the drug during encapsulation, washing or cross linking process, or reduced dissolution of the drug into solution during cross linking. Moisture contents of the samples produced at higher temperatures were expectedly, generally lower than those produced at ambient temperature. The moisture contents may also be correlated with swelling behaviour of the produced microcapsules when in contact with moisture and may be indicative of the level of drying of the samples or their moisture sorption capacities. This is consistent with an earlier study by some workers (30). The gels reached equilibrium swelling weights at shorter times in swelling media of higher pH than those of lower pH, whereas some of the gels never reached their equilibrium swelling weights throughout the period of the study. The generalized semi-empirical equation used to describe the swelling kinetics according to Harogoppad and Aminabhavi (31), Rathma and Gunasekaram (32) and Valencia and Pierih (33) is shown as equation 3 (Appendix). This equation is valid when $(SW_t / SW_\infty) < 0.6$. Based on the value of the exponent n, this equation has been used to distinguish three types of sorption behaviors – Case 1, Case 2 and Case 3 (anomalous) (34). Case 1 sorption is typified by n ~~0.5 and represents a perfect Fickian process, during which the rate of solvent penetration is slower, and hence being the rate determining step than the chain relaxation rate. For case 2 sorption, n = 1.0 i.e. the mobility of the penetrant is substantially faster than the chain relaxation rate and the solvent uptake is directly proportional to time. Case 3 or anomalous sorption occurs when $0.5 < n < 1.0$. In this case, the rate of penetrant mobility and segmental relaxation are comparable.

Therefore, the relative importance of solvent diffusion and polymer matrix relaxation effects can be analyzed by examining the exponent n of the power law. In the current study as shown in Table 3, the equation fits well in few of the cases where linear curves with high correlation coefficients were obtained. In the majority of the products where n is greater than 0.5 but less than 1.0, the process may be considered as anomalous sorption. The process involved in the swelling of the microcapsules prepared at 50^{O}C and 30 minutes cross linking time, is however clearly diffusion sorption as indicated by the value of 0.50 obtained for the constant n.

Swelling of pharmaceutical formulations is a great determinant of the release kinetics of the formulated drugs. Most formulations have to swell first, before bursting or disintegrating to discharge the drugs, a major pre-requisite for therapeutic efficacy of administered drugs, especially those passing through the gastrointestinal tract. The high swelling rate in the medium of acidic pH indicates that the formulations would swell extensively in the gastric region of the gastrointestinal tract which is composed mainly of

the gastric juice with a pH of about 1.2. In all the acidic and neutral swelling media, increasing the stirring (cross-linking) time correlated with reduction in the swelling capacities of the produced microcapsules. An earlier study has shown that about 30 min is optimal for the effective cross linking of chitosan and TPP (16).

It has previously been shown that the degree of cross-linking can be governed by the length and duration of cross-linking, and consequently the entrapment efficiency as well as overall microparticles characteristics (35). An earlier study has equally shown that about 30 minutes is optimal for the effective cross linking of chitosan and TPP (16) particularly if consideration is made for the drug loading efficiency. Previous studies (35,36) on cross-linked chitosan microparticles have conducted cross-linking at ambient temperature. It is therefore desirable to attempt to elucidate the resultant effect of increase in temperature on the degree of cross-linking of the prepared microparticles which would consequently influence the drug loading capacity. Hence, this study has also identified temperature of cross-linking as another important influencing factor for the outcome of cross-linking profile. This agrees with our earlier findings that higher degree of cross-linking was observed for the microparticles cross-linked at ambient temperature, followed by 40°C, and then 50°C. Increasing the temperature of cross-linking did not increase the drug loading efficiency shown in figure 2. The drug loading efficiency is seen to follow a decreasing trend in relation to the increasing temperature of cross-linking given the pyrimethamine intensified absorption depths at the band region 650 and 1500 earlier marked. Therefore the drug loading efficiency is highest at ambient temperature of cross-linking, followed by 40°C and 50°C respectively.

Increasing the temperature of production yielded microcapsules that had relatively low moisture content (Table 1), probably resulting to more active surfaces that more readily imbibed incoming penetrant (moisture) from the swelling medium, thereby increasing the swelling capacities. It is also probable that the microcapsules produced at the higher temperatures due to loss of inherent moisture content would swell more when introduced into the swelling medium.

From the results obtained in Figure 2 it could be shown that the microparticles resulted from the interaction between the positively charged amino group of chitosan and the negatively charged counter ion of TPP. Also, recorded absorption pattern would show that the spectrum of the drug-loaded microparticles is dominated by the chitosan / TPP complex bands. This could be as a result of the fact that the complex itself gives rise to intense absorption bands and both the host and guest molecules coincidentally absorb at most of the spectral regions. Also, there is the possibility of the influence of excess of free chitosan / TPP complex in the system. However the presence of pyrimethamine is obviously confirmed by the bands at 650 and 1500 cm^{-1} as displayed in Figure 2 (A).

The DSC curves (Figures 3 & 4) of the microparticle show an apparent combination of events of both chitosan, TPP and pyrimethamine. The absence of pyrimethamine melting peak in the drug loaded microparticles suggests absence of pyrimethamine isolated crystals which is consistent with efficient encapsulation processes. Also an increase in the thermal stability of pyrimethamine can be suggested since the drug loaded microparticles start to decompose at temperatures higher than in the isolated non loaded form. This behaviour has been previously described as an evidence of encapsulation (38,39).

Conclusion: Ionically cross-linked chitosan/tripolyphosphate microparticles containing pyrimethamine, an anti-protozoal were formulated. The spectral analyses confirm the occurrence of microencapsulation with the outcome of stable microparticles. These microparticles should be investigated further for clinical application as a controlled release matrix for the drug pyrimethamine.

Acknowledgement: The financial support of the Third World Academy of Sciences (TWAS)/UNESCO and the Conselho National de Desenvolvimento Cientifico e Technologico (CNPq-MCT), Brazil to Dr. Ibezim are gratefully acknowledged.

Appendix:

The percentage swelling of the pyrimethamine microcapsules was calculated from Equation 1:

$$S(\%) = \frac{W_e - W_o}{W_o} \times 100 \quad \text{Eqn 1}$$

Where W_e denote the weight of the gel microcapsules at equilibrium swelling and W_o is the initial weight of the microcapsules.

The amount of drug loaded was determined by measuring the absorbance of the solutions using the UV-Vis spectrophotometer at 200.99 nm wavelength and reference

to the standard Beer´s curve.

$$\text{Entrapment efficiency (\%)} = [W_a/W_t] \text{ X } 100 \quad \text{Eqn 2}$$

Where W_a is the actual pyrimethamine content and W_t is the theoretical pyrimethamine content.

The generalized semi-empirical equation used to describe the swelling kinetics is given below:

$$\frac{SW_t}{SW_\infty} = Kt^n \quad \text{Eqn 3}$$

Where K is a characteristic constant of the system, which is a function of the geometry of the bead and the diffusion constant.

References

1. Gasper de Araujo MV, Vieira EKB, Lazaro LC, Ferreira OP, Almeida LE, Barreto LS et al. Inclusion complexes of pyrimethamine in 2-hydroxypropyl-β-cyclodextrin: Characterisation, phase solubility and molecular modeling. Bioorg Medic Chem 2007;15:5752-9.
2. Bosch-Driessen LH, Verbraak FD, Suttorp-Schulten MSA, van Ruyven RLJ, Klok AM, Hoyng CB et al. Am J Ophthalmol 2002;134:34-40.
3. Anonymous. Pyrimethamine. (Internet Document. Available at http://en.wikipedia.org) accessed on 21/10/2007.
4. Anderson AC. Targeting DHFR in parasitic protozoa. Drug Discov Today 2005;10:121-8.
5. Vaamonde C, Contreras G, Diego G. Sulfonamides, sulfadiazine, trimethoprim-sulfamethoxazole, pentamidine, pyrimethamine, dapsone, quinolones. Clinical Nephrotoxins 2004;B:223-47.
6. Bodmeier R, Oh KH, Pramar Y. Preparation and evaluation of drug-containing chitosan beads. Drug Dev Ind Pharm 1989;15:1475–94.
7. Harris R, Paños I, Acosta N and Heras A. Preparation and characterization of chitosan microspheres for controlled release of tramadol. J Contr Release 2008;132:76-7.
8. Sinha VR, Singla AK, Wadhawan S, Kaushik R, Kumria R, Bansal K and Dhawan S Chitosan microspheres as a potential carrier for drugs. Int J Pharm 2004;274:1-33.
9. Shu XZ and Zhu KJ. The influence of multivalent phosphate structure on the properties of ionically cross-linked chitosan films for controlled drug release. Eur J Pharm Biopharm 2002;54:235-43.
10. Anal AK, Stevens WF and Remuñán-López C. Ionotropic cross-linked chitosan microspheres for controlled release of ampicillin. Int J Pharm 2006;312:166-73.
11. Shu XZ and Zhu KJA. Novel approach to prepare tripolyphosphate/chitosan complex beads for controlled release drug delivery. Int J Pharm 2000;201:51-8.
12. Yao KD, Peng T, Yin YJ, Xu MX, S-Rev JM. Microcapsules/microspheres related to chitosan. Polymer Rev. 1995;35:155–180.
13. Illum L. Chitosan and its use as a pharmaceutical excipient. Pharm Res 1-Heba AM. and Mosnad AF. Preparation of casein-chitosan microparticles containing diltiazem hydrochloride by an aqueous coacervation technique. Pharm Acta Helv 1998;73:187-92.
14. Mi FL, Sung HW, Shyu SS. Release of indomethacin from a novel chitosan microparticle prepared by naturally occurring cross-linker: examination of cross-linking and polycation-anionic drug interaction. J Appl Polym Sci 2001;81:1700-11.
15. Mi FL, Shyu S, Chen CT, Schoung JY. Porous chitosan microparticle for controlling the antigen release of Newcastle disease vaccine: preparation of antigen-adsorbed microparticle and in vitro release. Biomats 1999;20:1603-20.
16. Bumgardner JD, Wiser R, Gerard PD, Bergin P, Chestnutt B, Marini M, et al. Chitosan: potential use as a bioactive coating for orthopaedic and craniofacial/dental implants. J Biomater Sci Polym Ed 2003;14:429-38.
17. Khor E, Lim LY. Implantable applications of chitin and chitosan. Biomaterials 2003;24:2339-49.
18. Kumar MNVR. A review of chitin and chitosan applications. React Funct Polym 2000;46:1-27.
19. Martino AD, Sittinger M, Risbud MV. Chitosan: a versatile biopolymer for orthopaedic tissue-engineering. Biomaterials 2005;26:5983-90.
20. Senel S, McClure SJ Potential applications of chitosan in veterinary medicine. Adv. Drug Deliv Rev 2004; 56:1467-80.
21. Agnihotri SA, Mallikarjuna NN, Aminabhavi TM. Recent advances on chitosan-based micro- and nanoparticles in drug delivery. J Controlled Release 2004;100:5-28.
22. Sinha VR, Singla AK, Wadhawan S, Kaushik R, Kumria R, Bansal K, et al. Chitosan microspheres as a

potential carrier for drugs. Int J Pharm 2004;274:1-33.
23. Patashnik S, Rabinovich L, Golomb G. J. Preparation and evaluation of chitosan microspheres containing bisphosphonates. Drug Target 1997;4:371-80.
24. Jameela SR, Kumary TV, Lal AV, Jayakrishnan AJ. Progesterone-loaded chitosan microspheres: a long acting biodegradable controlled delivery system. Controlled Release 1998;52:17-24.
25. Prabaharan M, Mano JF. Chitosan-based particles as controlled drug delivery systems. Drug Deliv 2005;12:41-57.
26. Gupta KC, Jabrail FH. Glutaraldehyde and glyoxal cross-linked chitosan microspheres for controlled delivery of centchroman Carbohydr Res 2006;341:744-56.
27. Lim LY, Wan LSC and Thai PY. Chitosan microparticles prepared by emulsification and ionotropic gelation. Drug Dev Ind Pharm 1997;23:981-5.
28. Shu XZ, Zhu KJ and Song W. Novel pH-sensitive citrate cross-linked chitosan film for drug controlled release. Int J Pharm 2001;212:19-28.
29. Gunasekaran S, Ko S and Xiao L. Use of whey proteins for encapsulation and controlled delivery applications J Fd Engin 2007;83:31-40.
30. Harogoppad SB and Aminbhavi TM. Diffusion and sorption of organic liquids thorugh polymer membranes 5. Neoprene, styrene, butadiene rubber, ethylene propylene diene terpolymer and natural rubber veusus hydrocarbons (C8 – C16) Macromol 1991;24:2598-605.
31. Rathna GVN, Li J and Gunasekaran S. Functionally-modified egg white albumen hydrogels. Polym Internat 2004;53:1994-2000.
32. Valencia J, Pierola IF. Swelling kinetics of poly (N-vinyl imidazole-co-sodium styrenesulfonate hydrogels. J Appl Polym Scs 2002;83:191-200.
33. Lucht LM and Peppas NA. Transport of prenetrants in the macromolecular structure of coals, 5. Anomalous transport in pretreated coal particles. J Appl Polym Sc 1987;33:1557-66.
34. Ko JA, Park HJ, Hwang SJ, Park, JB and Lee JS. Preparation and characterization of chitosan microparticles intended for controlled drug delivery. Int J Pharm 2002;249:165-74.
35. Lee OS, Ha BJ, Park SN and Lee YS. Studies on the pH-dependent swelling properties and morphologies of chitosan/calcium alginate complexed beads. Macromol Chem Phys 1997;198:2971-6.
36. Araujo MVC, Viera EK, Lazaro GS, Conegero LS, Ferreira OP, Almanda CE, Barreto LS, Bezerra do Costa N and Gimenez IF. Inclusion complexes of pyrimethamine in 2-hydroxypropyl-β- cyclodextrin: Characterization, phase solubility and molecular modeling. Bioorg Medic Chem 2007;15:5752-9.
37. Veiga MD, Merino M, Fernandez D, Lozano R. Characterization of some cyclodextrin derivatives by thermal analysis. J. Therm Anal Calorim 2002;68:511-6.
38. Liu Y, Chen GS, Chen Y, Lin J. Inclusion complexes of azadirachtin with native and methylated cyclodextrins: solubilization and binding ability. Bioorg Medic Chem 2005;13:4037-42.