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Microspheres of Guar gum-grafted- polyacrylamide modified by hydrolysis: Evaluation for controlled drug delivery

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ABSTRACT

Guar gum-g-poly(acrylamide) microspheres were made by emulsion method using MBA as crosslinker. The microspheres were subsequently modified by alkaline hydrolysis to generate carboxylic groups. The modified microspheres have been characterised by SEM, FTIR, Elemental analysis and TGA techniques. The drug entrapment efficiency, particle size distribution and swelling behaviour of the microspheres were studied. The drug entrapment efficiency of the microspheres was found to be 77.7 % and the average particle size varied between 50–100 µm. The drug release data were analysed using Korsmeyer–Peppas model. The value of swelling exponent n was found to be > 0.5 indicating anomalous (non-Fickian) diffusion as the mechanism of drug release.

Keywords: Microspheres, Guar gum, polyacrylamide, hydrolysis, drug release.

1. INTRODUCTION

Polymer systems in the form of microspheres are attractive materials for biomedical and pharmaceutical applications [1, 2]. These systems act as reservoirs of therapeutic agents, with spatial and temporal control of the drug leading to desirable therapeutic outcomes. Natural polymers are often preferred to synthetic materials in controlled release formulations due to their non-toxicity, low cost, free availability and biodegradability. However, many natural polymers have some inherent disadvantages such as poor mechanical strength and microbial contamination. To overcome these problems, efforts have been made to develop matrices by combining natural polymers with synthetic ones. Graft copolymerization is an easier method to modify the structure of natural polymers for biomaterial applications [3].

Polysaccharides are a class of natural polymers which find extensive use in food industry as gelling agents and in the pharmaceutical industry as matrix materials for the encapsulation of living cells and drugs. Among such polymers, Guar gum(GG) has attracted much attention recently. It is a high molecular weight, hydrocolloidal, hetero-polysaccharide composed of galactan and mannan units. Guar gum has been modified by derivatization, grafting and network formation to improve its property profile for a wide spectrum of end-uses[4,5]. Grafting of methacrylamide, acrylonitrile, acrylic acid and acrylamide on guar gum, using different initiators has been reported [6]. Use of microwaves and high energy radiation (Co ^{60} γ-radiation) for grafting of acrylamide on guar gum has been reported [7].

Polyacrylamide is a water-soluble synthetic polymer with a hydrophobic main chain and a hydrophilic side group. Crosslinked polyacrylamide is a well known hydrogel, whose swelling nature is not very sensitive to pH or to the presence of electrolytes. The advantage of amide functional groups is that, it can be used to introduce required degree of ionic groups in the gel, imparting pH sensitivity to the gel. The hydrogel of natural polymers modified with polyacrylamide finds application in drug delivery and other fields [8,9]. Studies on water transport and drug release from cross-linked polyacrylamide grafted guar gum hydrogel microspheres for the controlled release application has been reported [10].

The objective of the present study is to obtain guar gum- grafted- polyacrylamide in the form of microspheres and to modify the microspheres by hydrolysis. The modification is expected to generate carboxylic groups on surface of microspheres which would impart the microspheres a pH sensitive swellability. This system has been evaluated as a matrix material in

tablet formulations for pH dependent drug delivery. The experimental parameters governing the microsphere yield, particle size and drug entrapment efficiency have been investigated. The pH dependency of swelling has been measured. The release profile of the entrapped drug, namely, metacloproamide hydrochloride (MH) has been studied in detail and the results have been discussed.

2. MATERIALS AND METHODS

Acrylamide(AAm) and N,N'-methylene-bis-acrylamide (MBA) (s.d.fine, Mumbai, India), Guar gum (GG) and potassium persulfate (KPS) (Merck,Mumbai) were used as received. Acrylamide was purified by recrystallisation from chloroform before use. All other reagents were of analytical grade. Double distilled water was used in the preparation of hydrogels and for swelling studies.

2.1. Preparation of guar gum-g-poly(acrylamide) (GG-PAAm) microspheres

GG-PAAm microspheres were prepared by W/O emulsion method. Briefly, 0.1g of GG was dispersed in 20mL water in a closed container and stirred overnight. 0.028 mol AAm and 7.39 mmol KPS were dissolved in 5mL water and the mixture was added to the GG solution under stirring and mixed uniformly for 1h. 7.39 mmol of MBA was dissolved in 5mL water and added to above solution. This solution was added slowly to the light liquid paraffin (200 g) containing 1% (w/w) Tween-80 under constant stirring at 3000-4000 rpm for 10 min. The temperature was slowly increased to 60°C and stirring was continued for 5 h. Hardened microspheres were separated by filtration and washed with petroleum ether. The microspheres were dried at 50°C for 24 h and stored in a dessicator for further use.

2.2. Alkaline hydrolysis of GG-PAAm microsphere

To a known quantity of dry microspheres, 1N NaOH (20mL) was added and the mixture was maintained at 90-100°C for 1hr. It was then allowed to cool to room temperature and neutralized to pH-8 by addition of aqueous acetic acid (10%).The resulting mixture was added to methanol (200mL). After an hour the microspheres were filtered off and dried at 50 °C under vacuum for 5hrs and stored. It was designated as guar gum-poly(acrylic acid) (GG-PAA) microspheres.

2.3. Loading of MH

50mg dry GG-PAA microspheres were soaked for 24hrs in 10 mL aqueous solution containing 50mg of MH drug. Later the excess amount of drug solution was drained out and the loaded microspheres were washed repeatedly with water for removing the surface adhered drug. They were dried at 50 °C in vacuum oven till they attained constant weight. They were used for drug release studies.

2.4. Estimation of drug loading

Loading efficiency of the microspheres was determined spectrophotometrically. Drug-loaded microspheres were accurately weighed (50mg), ground to fine powder using mortar and pestle and placed in 50 mL of buffer 7.4 and stirred vigorously for 24 h to extract the drug from the microspheres. The solution was filtered, diluted suitably and assayed by UV spectrophotometer (Shimadzu 1601) at 273nm. The % encapsulation efficiency was calculated using the following equation:

$$\text{Entrapment efficiency}\ (\%) = \left(\frac{\text{actual drug content}}{\text{theoretical drug content}}\right) \times 100$$

2.5. In vitro release profile

To obtain information about the possible mode of action of the proposed drug delivery system in the human body, it is often more convenient to perform the same studies in an environment almost similar to that in the body. Hence, for the purpose of carrying out the drug release study in an in vitro manner, two buffer solutions, of pH 1.2 and 7.4, were used and the drug release studies were carried out in these media using USP-1 basket type apparatus (Electrolab TDT-08L Dissolution Tester). 50mg of drug-loaded microspheres were taken in the basket and immersed into the dissolution tank containing 900ml of the buffer. The basket was maintained at 100 rpm at 37±1℃. 5ml of the sample was withdrawn at predetermined time intervals and replaced with equal volume of fresh dissolution medium. These aliquots were diluted suitably with corresponding buffer and the amount of the drug released was estimated. The percentage cumulative drug release (CDR) was calculated using the following equation:

$$\%\text{CDR} = \frac{\text{Amount of drug released}}{\text{Amount of drug loaded}} \times 100$$

2.6. Characterization of GG-PAAm and GG-PAA microspheres

2.6.1. FTIR analysis

The FTIR spectra of GG, GG-PAAm and GG-PAA samples were recorded as KBr pellets on a FTIR spectrophotometer (Perkin-Elmer, USA) in range of 4000–400 cm^{-1}.

2.6.2. Elemental analysis

Elemental analyses of GG, AAm, GG-PAAm and GG-PAA samples were carried out using a

Vario EL III CHN analyzer (Germany) and percentages of carbon, hydrogen and nitrogen in these samples were determined.

2.6.3. Determination of neutralization equivalent (N.E.) of GG-PAA sample

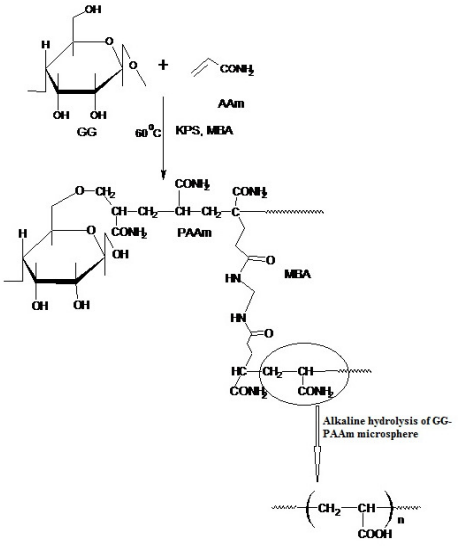
The neutralization equivalent (N.E.) is the equivalent weight of the acid as determined by titration with standard base [13]. In order to confirm the conversion of amide functionality to carboxylic acid, neutralization equivalent of GG-PAA sample was determined in the following way: The volume (Y mL) of NaOH (Z (N)) required for complete neutralization of X gm of the sample was determined. The equivalent weight of the sample was determined according to the expression,

$$\text{Neutralization equivalent(N.E.)} = \frac{X \times 100}{Y \times Z}$$

2.6.4. Thermal analysis

TGA of GG, GG-PAAm and GG-PAA microspheres were carried out on SDT Q600 V20.9 (Japan) thermogravimetric analyser. The samples were heated from zero to 700°C, under nitrogen atmosphere, at a rate of 5 °C / minute.

DSC of GG-PAAm and GG-PAA microspheres was recorded on Q20 V24.4 Build 116 (USA) calorimeter by heating the samples under nitrogen atmosphere from 40 to 500 °C at a rate of 10 °C / minute.



Scheme - 1: Formation of GG-PAAm and GG-PAA microspheres.

3. RESULTS AND DISCUSSION

The synthesis of GG-PAAm network was achieved by polymerization of AAm in the presence of GG by free radical polymerization along with simultaneous crosslinking using MBA. The grafting of AAm is expected to occur at the active site of GG followed by crosslinking of the chains. As the reaction is carried out in organic medium containing a surfactant, the product is obtained in the form of microspheres.

Purified microspheres were subjected to saponification using NaOH. Alkaline hydrolysis resulted in the GG-PAA network with few of the amide groups converted to carboxylic acid groups. The formation of GG-PAAm microspheres and alkaline hydrolysis to form GG-PAA microspheres is presented in scheme 1.

3.1. FTIR characterization of GG-PAAm and GG-PAA microspheres

The FTIR spectra of GG, GG-PAAm and GG-PAA samples have been compared in Figure 1. FTIR spectrum of GG(Figure 1a) exhibits characteristic absorption bands at 3431.8 and 2925.4 cm^{-1} attributed to O-H and C-H stretching vibrations. Additional characteristic absorption bands of GG appear at 1418 and 1023 cm^{-1} attributed to C-H bending and O-H bending respectively. In the spectrum of GG-PAAm (Figure 1b), the sharp peak that appeared at 3417. 2 cm^{-1} is due to O–H stretching. Besides, peaks at 2920 cm^{-1} &1623 cm^{-1} appear due to C-H stretching of GG and C=O stretching of amide groups. In the spectra of GG-PAA (Figure 1c) peaks appear at 3428.7 and 2924.3 cm^{-1} corresponding to O-H and C-H stretching of GG respectively. The peak at 2854 cm^{-1} correspond to C-H stretching of polyacrylamide chains and 1457 cm^{-1} correspond to $-CH_2$ bending vibration respectively. Moreover new peak observed at 1743, 1674 & 1527cm^{-1} are attributed to C=O stretching of carboxylic acid, -C=O stretching of MBA & N-H stretching of amide groups respectively. These observations confirm the amide to carboxylic acid conversion in the GG-PAAm network on alkaline hydrolysis.

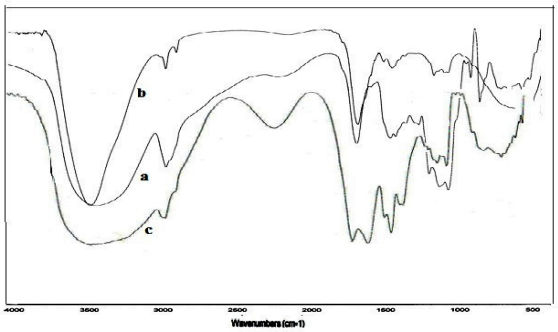


Figure - 1: FTIR spectra of a) GG b) GG-PAAm and c) GG-PAA samples

3.2. Determination of Neutralization equivalent (N.E.) & Elemental analysis of GG-PAAm and GG-PAA samples

Upon hydrolysis of the amide groups, the molecular weight of the polymer would remain nearly the same (as $-NH_2$ is replaced by -OH group) but carboxyl groups will be generated. The

N.E value of GG-PAA was found to be 222, indicating a high degree of conversion of amide groups to acid groups. The conclusive proof for this result comes from the elemental analysis data shown in Table 1. The % of N of GG-PAA sample reduces by 70% compared to GG-PAAm sample(from 13% to 4%) without much variation in the % of C and H, indicating large conversion of $-CONH_2$ to $-COOH$ groups.

Table - 1: Elemental analysis data

Sample code	% N	% C	% H
GG	0.8	38.08	6.54
AAm	18.78	49.76	6.7
GG-PAAm	13.06	43.85	7.05
GG-PAA	4.06	42.31	7

3.3. Thermogravimetric analysis (TGA)

The TGA thermograms of GG, GG-PAAm & GG-PAA are shown in Figure 2. The observed initial mass loss up to 120 °C in figure 2a &b may be due to the presence of moisture, solvents, the unreacted cross-linking agents or the monomers. In case of GG(Figure 2a), a sharp mass loss of about 50–55% is observed between 230 and 350°C and this may be attributed to the loss of hydroxyl group of GG as water molecules. The GG-PAAm microspheres (Figure 2b) indicates very little weight loss initially, but % loss increases gradually. The degradation is gradual and about 35% weight is lost at 350 °C. A further increase in the rate of weight loss is observed in the range 350-450 °C, which may be due to loss of NH_2 in form of ammonia. The final 30% weight loss occurs in the range 500-600 °C leaving only 5-10% of residual mass. The degradation pattern of GG-PAA microspheres (Figure 2c) appears to be very similar to GG-PAAm up to 200 °C, but the weight loss is relatively less in the former. Only 30% weight is lost up to 300 °C and an additional 25% loss occurs in the range 350-550 °C. This could be due to the anhydride decomposition with loss of CO_2 and a residual mass of 30-35% is still present at 600 °C compared to 30-32% in GG-PAAm.

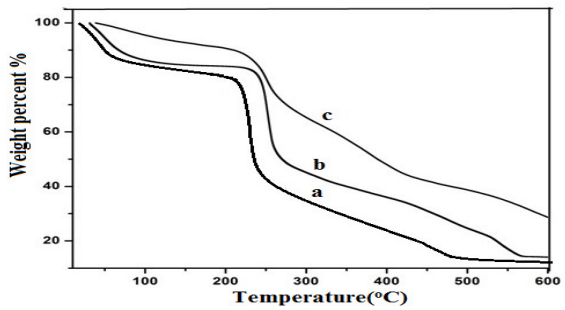


Figure - 2: TGA thermograms of (a) GG, b) GG-PAAm & (c) GG-PAA microspheres

3.4. Differential scanning calorimetry (DSC)

The DSC thermograms of GG, GG-PAAm and GG-PAA microspheres are presented in Figure 3. In Figure 3a, GG exhibited endothermic peak around 50-100 °C due to the presence of moisture, the unreacted crosslinking agents or the monomers. The exothermic peak around 300-325 °C is due to the loss of hydroxyl group of GG as water molecules. In Figure 3b, GG-PAAm exhibited endothermic peak corresponding to melting transition of PAAm at 237 °C [14] which disappears in GG-PAA samples (Figure 3c) indicating the $-CONH_2$ to $-COOH$ conversion in the sample. This observation further confirms the formation of $-COOH$ in segments of grafted PAAm chains.

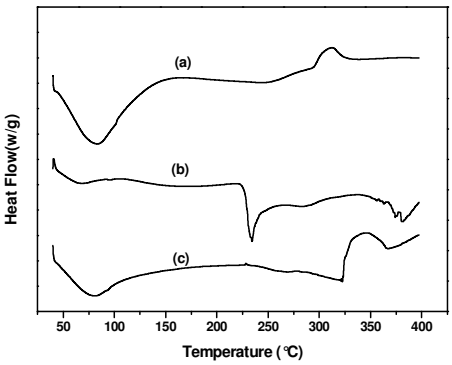


Figure - 3: DSC thermograms of (a) GG, (b) GG-PAAm & (c) GG-PAA Microspheres.

3.5. Scanning Electron Microscopic (SEM) Analysis

The GG-PAA microspheres are spherical in shape and are agglomerated as indicated by the SEM photograph shown in Figure 4. The microspheres have a mean diameter ranging from 50 to 100µm. The surface appears to be highly porous.

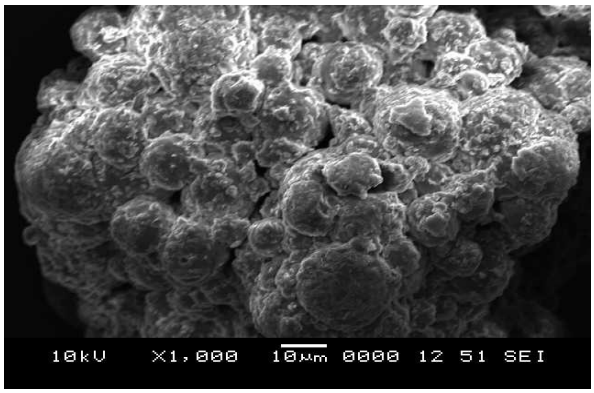


Figure - 4: SEM images of GG-PAA microspheres

3.6. Swelling Studies

The effect of pH of the medium on the swelling behaviour of the microspheres was

studied by maintaining the pH of the medium at 1.2 and 7.4. The swelling behaviour exhibited by the representative of GG-PAA is shown in Figure 5. The swelling is found to be much lower at pH 1.2 when compared to pH 7.4. If the pH of the external medium is above the pKa of PAA, ionization of the carboxylic acid groups occur which results in a more hydrophilic polymer network leading to higher absorption of water. Also, the increase in the ionization of functional groups at a pH greater than pKa causes electrostatic repulsion between the chain segments leading to chain expansion, which in turn affects water absorption. As a result the microspheres at higher pH swell by relaxation-controlled mechanism.

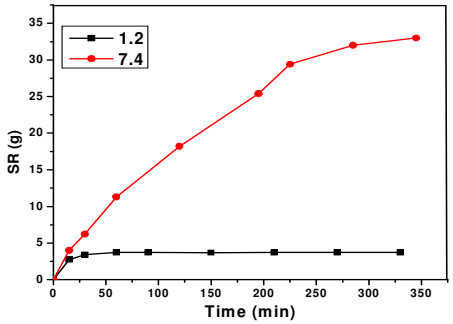


Figure - 5: The swelling data for the GG-PAA microspheres under different pH conditions.

3.7. Drug release studies from GG-PAA microspheres

When the drug-loaded dry microspheres come in contact with a solvent, the microspheres swell and the entrapped drug passes into the external receiving medium. Depending on the mechanism of the swelling process, the drug release may be Fickian or non-Fickian. In the present work, the release of MH from the microspheres was studied at pH 1.2 and 7.4 at the physiological temperature of 37±1°C. The results depicted in figure 6 clearly indicate that the drug loaded sample releases a higher amount of MH in the medium of pH 7.4, and a comparatively low amount of the drug is found to be released at pH 1.2. The quantity of the total drug released from the microspheres in the release medium of pH 1.2 and 7.4 was 22% and 98.5%, respectively. The results indicate that the release of the drug from these microspheres depends on swelling.

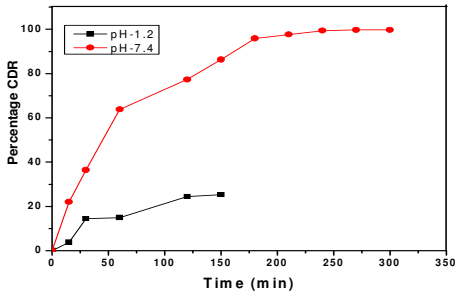


Figure - 6: Release profiles of MH from GG-PAA microspheres.

3.7. Release Kinetics of MH from GG-PAA microspheres.

In order to describe the kinetics of the release of the drug in from GG-PAA microspheres, the release data are fit into various equations. The zero order rate equation describes the systems where the release rate is independent of the concentration of the drug. The First-order equation describes the release from systems where release rate is dependent on the concentration of the drug in the matrix. The Higuchi square root equation [11] describes the release from systems where the solid drug is dispersed in an insoluble matrix. The equation that best fits the release data is selected based on the correlation coefficient (R^2) value of fit. The R^2 values obtained for the various fits made with the present data are given in table 2.

When the dissolution data obtained at pH-7.4 were plotted in accordance with the Zero-order equation, the plots are curvilinear, suggesting that the release process is not a zero-order process(Figure 7a). When the dissolution data were plotted in accordance with the first-order equation, that is the log (% drug remained) vs. time (Figure 7b), a linear relationship is obtained with better R^2 values than for Zero-order fit .This indicates that the amount of drug released is dependent on the matrix drug load. To evaluate the drug release mechanism from the microspheres, plots of percent drug released vs $t^{1/2}$, as per Higuchi's equation were constructed (Figure 7.0 (c)). These plots were found to be non-linear with correlation coefficient value of 0.874 indicating that the drug release from the matrix was diffusion-controlled [15]. When the release data were analyzed as per Korsemeyer and Peppas's equation [12] (Figure 7.0(d)), a linear

Table - 2: Drug Release Parameters at pH 7.4.

Sample code	% Drug loading	Correlation coefficient (R^2) value				(n) value Korsemeyer
		Zero order	First order	Higuchi's square root	Korsemeyer	
GG-PAA	77.7	0.737	0.989	0.874	0.993	0.509

relationship was obtained with high R^2 values than for other models indicating that the release is an apparent Korsemeyer process. The release exponent 'n' was $\geq$ 0.5 for the present system, indicating an anomalous diffusion as the release mechanism.

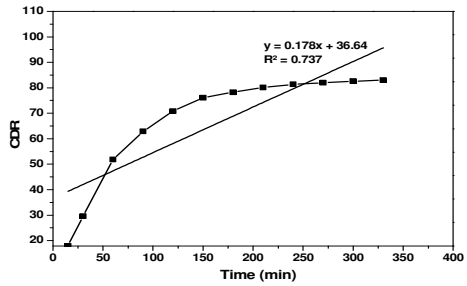


Figure - 7(a): Drug release data (pH 7.4) plotted in accordance with Zero-order equation.

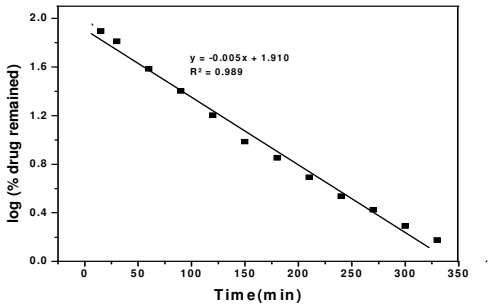


Figure - 7(b): Drug release data (pH 7.4) plotted in accordance with First-order equation.

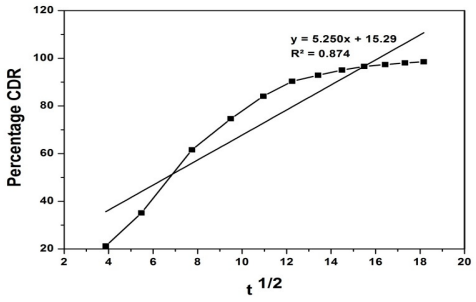


Figure - 7(c): Drug release data (pH 7.4) plotted in accordance with Higuchi equation.

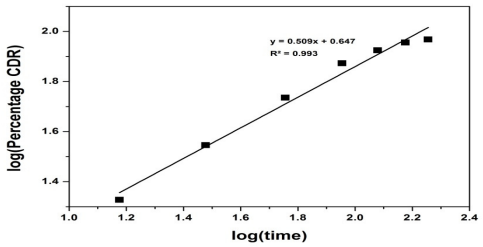


Figure - 7(d): Drug release data (pH 7.4) plotted in accordance with Korsemeyer equation.

4. CONCLUSIONS

Crosslinked GG-grafted-PAAm was obtained in the form of microspheres by emulsion method using MBA as crosslinker. These microspheres were modified by alkaline hydrolysis, wherein amide groups are partially converted to carboxylic groups. The microspheres have been observed to exhibit a high pH responsive swelling behaviour and good drug entrapment efficiency for of the chosen drug, metacloproamide hydrochloride. The drug loaded microspheres exhibit pH dependent release behaviour. The release process followed Korsemeyer model indicating the dependence of release on matrix-drug load. The release exponent 'n' is found to be > 0.5 indicating anomalous diffusion as the mechanism of drug release from the microspheres.

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5. REFERENCES

1. Freiberg S and Zhu X X. Polymer microspheres for controlled drug release. **International Journal of Pharmaceutics.** 2004; 282; 1–18.
2. Kumbar SG and Aminabhavi TM. Synthesis and characterization of modified chitosan microspheres: Effect of the grafting ratio on the controlled release of nifedipine through microspheres. **Journal of Applied Polymer Science.** 2003; 89:2940–2949.
3. Shelke NB and Aminabhavi TM. Synthesis and characterization of novel poly (sebacic anhydride-co-Pluronic F68/F127) biopolymeric microspheres for the controlled release of nifedipin. **International Journal of Pharmaceutics.** 2007; 345: 51–58.
4. Xiuyu, Li, Wenhui, Wu. Weiqi and Liu. Synthesis and properties of thermo-responsive guar gum/poly(*N*-isopropylacrylamide) interpenetrating polymer network hydrogels **Carbohydrate Polymers**. 2008; 71: 394-402.
5. Behari K, Kumar R, Tripathi M and Pandey PK. Graft copolymerization of methacrylamide on to guar gum using a potassium chromate/malonic acid redox pair. **Macromolecular Chemistry and Physics.** 2001**;** 202: 1873-1877.
6. Pandey PK, Srivastava A, Tripathy J and Behari K. Graft copolymerization of acrylicacid onto guar gum initiated by vanadium (V)–mercaptosuccinicacid redox

pair. **Carbohydrate Polymers.** 2006; 65: 414 420.

7. Biswal J, Virendra K., Bhardwaj YK, Goel NK., Dubey KA, Chaudhari CV and Sabharwal S. Radiation-induced grafting of acrylamide onto guar gum in aqueous medium: Synthesis and characterization of grafted polymer guar-g-acrylamide. **Radiation Physics and Chemistry.** 2007; 76: 1624–1630.
8. Hiremath JN and Vishalakshi B. Effect of Crosslinking on swelling behaviour of IPN hydrogels of Guar Gum & Polyacrylamide. **Der Pharma Chemica.** 2012; 4: 946- 955.
9. Mohanan A, Vishalakshi B and Ganesh S. Swelling & metal ion adsorption characteristics of radiation synthesized stimuli response PAAm-KC semi IPN hydrogels. **Separation Science and Technology**, 2011; 46: 2041-2048.
10. Soppirnath Kumaresh S and Aminabhavi Tejraj M. Water transport and drug release study from cross-linked polyacrylamide grafted guar gum hydrogel microspheres for the controlled release application. **European Journal of Pharmaceutics and Biopharmaceutics.** 2002; 53:87-98.
11. Higuchi, T. Rate of release of medicaments from ointment bases containing drugs in suspension. **Journal of Pharmaceutical Sciences**. 1961; 50: 874-875.
12. Korsemeyer R, Gurny R and Peppas N. Mechanisms of solute release from porous hydrophilic polymers. **International Journal of Pharmaceutics**.1983; 15: 25-35.
13. Tripathy T and Sing RP. High performance flocculating agents based on partially hydrolyzed sodium alginate-g-polyacrylamide. **European Polymer of Journal.** 2000; 36: 1471-1476.
14. Mukhles, Sowwan. Sami, Makharza, Wadie Sultan., Jamal ghabboun, Musa Abu Teir and Hasan Dweik Analysis, characterization and some properties of polyacrylamide- Ni (II) complexes. **International Journal of Physical Sciences.** 2011; 6: 6280-6285.
15. Mukesh C. Gohel, Maulik K. Panchal and Viral V. Jogani Noval mathematical method for quantitative expression of deviation from the Higuchi model. **AAPS PharmSciTech.** 2000; 1:43–48.