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Formulation Development and Characterization of 5-Fluorouracil Based Microbeads Using Hydroxypropyl-β-Cyclodextrin for Innovative Drug Delivery

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***SUMMARY*. The aim of the study was to formulate microbeads of hydroxypropyl-β-cyclodextrin (HP-β-CD) using sodium alginate (SA) containing 5-fluorouracil (5-FU) by emulsion crosslinking technique. The microbeads were prepared by varying the concentration of hydroxypropyl-β-cyclodextrin (HP-β-CD) and sodium alginate (SA). The prepared microbeads were evaluated for swelling degree, drug entrapment, scanning electron microscopy (SEM), Fourier transforms infrared spectroscopy (FTIR), X-ray diffraction (XRD) and differential scanning calorimetry (DSC). SEM confirms the slight rough nature of microbeads. No significant drug polymer interactions were observed in FTIR studies. XRD and DSC revealed amorphous nature of drug after being entrapped. Gas chromatography confirms the absence of glutaraldehyde residue. The drug release shows excellent sustained drug release pattern.**

RESUMEN. El objetivo del estudio fue formular microperlas de hidroxipropil-β-ciclodextrina (HP-β-CD) utilizando alginato sódico (SA) que contiene 5-fluorouracilo (5-FU) mediante la técnica por emulsión de entrecruzamiento. Las microperlas se prepararon mediante la variación de la concentración de hidroxipropil-β-ciclodextrina (HP-β-CD) y alginato sódico (SA), se evaluaron el grado de hinchamiento y el atrapamiento de drogas y se analizaron por microscopía electrónica de barrido (SEM), espectroscopia infrarroja por transformadas de Fourier (FTIR), difracción de rayos X (DRX) y calorimetría diferencial de barrido (DSC). SEM confirma el carácter liviano de las microesferas. No se observaron interacciones significativas del polímero y la droga en los estudios de FTIR. XRD y DSC revelaron la naturaleza amorfa del medicamento después de ser atrapado. La cromatografía de gases confirma la ausencia de residuos de glutaraldehído. El fármaco muestra un excelente patrón de liberación sostenida.

INTRODUCTION

The use of biodegradable polymers played an integral role in improving human health . These polymers shows many superior properties over other conventional dosage forms like improved efficacy, better therapeutic effect and decreased toxicity of drug after being entrapped [3]. Multiple dosage units such as microspheres and microbeads have attained high popularity as oral drug delivery systems because of uniform drug absorption, distribution, reduced local irritation and elimination of undue side effects when compared to other dosage forms. Microbeads appears to be a small, free flowing carriers containing dispersed particles of drug either in solution or crystalline forms allowing a sustained release effect deprived of major side effects [4]. Various polymers used for microbeads formation includes chitosan, sodium alginate, gelatin and chondroitin sulphate [5].

β-cyclodextrin and its hydrophilic derivatives, have received a considerable attention due to their multifunctional characteristics. A noticeable increase in drug solubility is frequently observed due to for-

KEY WORDS: crosslinking, 5-fluorouracil, FTIR, hydroxypropyl-β-cyclodextrin, microbeads.

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mation of water soluble drug-polymer complexes. Water soluble polymers at low concentration are known to interact with cyclodextrin and increase the complexing abilities of cyclodextrins, thus consequently enhance the availability of drugs in aqueous cyclodextrin solutions. The interaction in the formed system results in physicochemical changes and solubility of drug increases [8-10]. Hydroxypropyl-β-cyclodextrin (HP-β-CD) is non-toxic, biocompatible, relatively inexpensive and more appropriate to encapsulate variety of molecules. Combination of sodium alginate (SA) and HP-β-CD were used for solubilization of hydrophobic drugs [11]. Calcium alginate beads linked covalently with α-cyclodextrin was already prepared to examine their ability as a supporting matrix for degrading bacteria [12]. 5-fluorouracil (5-FU), the major antimetabolite used against variety of solid tumors, is usually given intravenously due to its erratic and unpredictable absorption form gastrointestinal tract. It is sparingly soluble in water so it is important to improve its solubility and dissolution by using HP-β-CD.

The present study describes a simple method of preparing microbeads by emulsion crosslinking technique. HP-β-CD has been evaluated to probe its enhancing effect on solubility and stability of 5-FU. The physicochemical characteristics of prepared microbeads were determined (*i.e.*, encapsulation efficiency, drug loading, *in vitro* release) showing a novel drug delivery system.

MATERIALS AND METHODS

Materials

5-FU was a gift sample from Roche Laboratories Pvt. Ltd. Karachi, Pakistan. SA and HP-β-CD was purchased from Sigma-Aldrich, Germany. Glutaraldehyde 25% v/v was obtained from Sigma-Aldrich Germany. Deionized distilled water was obtained from Pharmaceutics research Lab of Faculty of Pharmacy, BZU Multan, Pakistan.

Solubility Studies

The solubility studies for pure drug 5-FU was carried out in Phosphate buffer solution of pH 1.2, 6.8, and 7.4. In each case excess amount of drug was added to 10 mL of solvent and agitation was carried out at 37 °C using a rotary test tube shaker for 24 h. After equilibrium, samples were filtered through 0.45 μm Millipore filters, diluted with phosphate buffer and analyzed to determine the content of 5-FU by using UV-visible spectrophotometer at 266 nm.

Preparation of microbeads containing 5-FU

A stable complex of HP-β-CD and 5-FU (200 mg) was formed in 8 mL distilled water preheated up to 60 °C. The presence of HP-β-CD favors the rapid drug dissolution to form a stable complex as described previously by several authors [13-16]. Different formulations coded as NB1 to NB9 were prepared with varying concentration of HP-β-CD. The concentration of drug was kept constant and mentioned in **Table 1**.

Formulation code	HP-β-CD (g)	Sodium alginate (g)	Cross-linker (mL)
NB1	0.09	0.01	1.5
NB2	0.08	0.02	1.5
NB3	0.07	0.03	1.5
NB4	0.18	0.02	1.5
NB5	0.16	0.04	1.5
NB6	0.14	0.06	1.5
NB7	0.27	0.03	1.5
NB8	0.24	0.06	1.5
NB9	0.21	0.09	1.5

Table 1. Composition of complex loaded microbeads having the constant concentration of 0.2 g 5-FU.

SA was dissolved separately in 7 mL of distilled water by stirring overnight. The aqueous complex was then added to a polymeric solution of SA by constant stirring at 1500 rpm for 30 min. The above solution was added drop wise to magnetically stirred liquid paraffin containing 1 g of tween 80 and 0.14 g of magnesium stearate to obtain a stable emulsion. 1.5 mL of 25% v/v aqueous solution of glutaraldehyde with 0.1 M HCl used as a cross-linker. The gel microbeads developed were washed with n-hexane to remove the excess of oil and unreacted glutaraldehyde. Drying was done under vacuum and gel beads were stored in desiccators. The crosslinked microbeads were shown in **Fig. 1**.

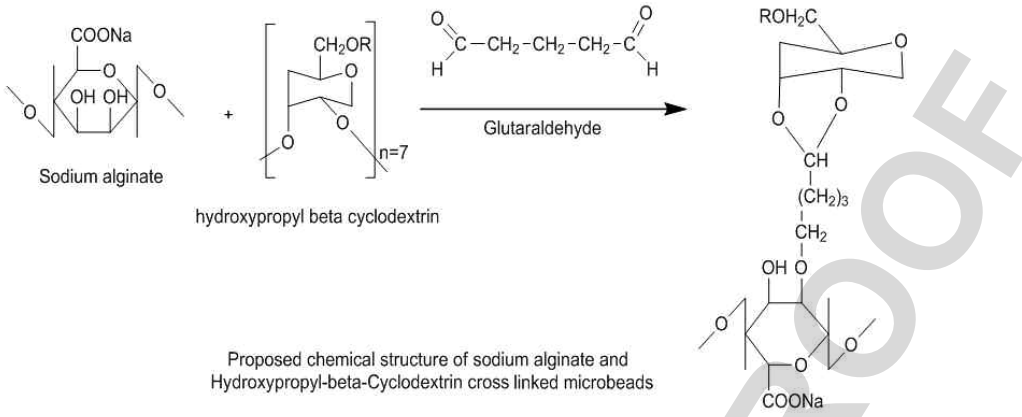


Figure 1. Formation of crosslinked microbeads containing 5-fluorouracil

Scanning electron microscopy (SEM)

The shape and surface morphology of prepared microbeads was observed using SEM (Jeol, JSM-5910, Japan). Microbeads were fixed with carbon-glue and coated with gold using gold sputter coater to avoid charging. Samples were observed by SEM under 5 kV energy.

X-ray diffraction analysis (XRD)

X-ray diffraction data was obtained using (Bruker Kahlsruhl, Germany) to determine crystallinity of drug. A plastic sample holder was filled with powder of pure drug, drug containing microbeads and empty microbeads. These samples were than scanned over the range of 5-50° 2θ at the rate of 1° 2θ/min by using kα radiation source. All the prepared samples were analyzed in triplicate.

Fourier transforms infrared spectroscopy (FTIR)

FTIR spectra of prepared microbeads, empty microbeads and pure drug were obtained using ATR-FTIR (shimadzu, Germany) in the range of 4000-650 cm^{-1}. Samples were grounded thoroughly and analyzed by attenuated total reflectance (ATR-FTIR).

Differential scanning calorimetry (DSC/TGA)

DSC analysis was carried out on pure drug, microbeads containing drug and empty microbeads using DSC Q 1600, TA instruments, USA. Samples were placed in aluminum pan and experiment run at 10 °C/min heating rate under nitrogen atmosphere. TGA analysis was performed using USA model 1600 under inert nitrogen atmosphere as a carrier gas. TGA curves were recorded up to 500 °C. Initial decomposition temperatures were recorded from thermograms.

Test for glutaraldehyde residue

The residue of glutaraldehyde present in the prepared microbeads was determined using gas chromatography technique. An aqueous extract of prepared microbeads was injected in to (GC model 2010, Shimadzu, Japan) fitted with a column DB-5MS Agilent for the detection of glutaraldehyde residue. The detector temperature was fixed at 200 °C with flow rate of helium gas 1 mL/min [17].

Swelling studies

Swelling studies were performed by taking 10 mg 0f microbeads. Microbeads were soaked in phosphate buffer of pH 7.4 to perform swelling experiments. Microbeads were allowed to swell at 37 °C upto 24 h. Excess of solvent was carefully removed using blotting filter paper. Swelled microbeads were weighed. The weight % water uptake was calculated according to Eq. [1] [18]:

$$\%\ Water\ uptake = \frac{W1-W2}{W2} \times 100 \quad [1]$$

where W1 is weight of swollen microbeads and W2 weight of dried microbeads.

Estimation of drug loading and encapsulation efficiency

About 200 mg of microbeads containing 5-FU were taken and crushed in a glass mortar and pestle. These microbeads were soaked in to 25 mL of phosphate buffer at pH 7.4 by constant stirring for 24 h. Aliquots were taken out and filtered. The concentration of drug was measured at 266 nm using UV- Spec-

trophotometer. Readings were taken in triplicate to calculate % DL and % EE by using Eqs. [2] and [3], respectively [19]:

$$\%\ Drug\ Loading = \frac{Mt}{Mp} \times 100 \quad [2]$$

$$\%\ Encapsulation = \frac{DL}{TL} \times 100 \quad [3]$$

where Mt = total weight of drug extracted from microbeads, M_p = weight of microbeads, DL = actual drug loading, and TL = theoretical drug loading.

In vitro drug release

In vitro drug release was carried out using USP dissolution apparatus, where 100 mg of samples was placed inside a dialysis bag having 4 mL of dissolution medium. The release medium is HCl buffer solution of pH 1.2 and phosphate buffer solution of pH 6.8 and 7.4 at 37.5 ± 0.5 °C. A sample was collected from the release medium at regular intervals and replaced by fresh dissolution medium. The amount of drug released was determined by using UV-Visible spectrophotometer (Perkin Elmer, USA) at 266nm. All experiments were performed in triplicate form [20].

Drug release kinetics

Drug release kinetics was performed to analyze in vitro data by using different kinetic models. Zero order equation describes that release of drug is independent of concentration as shown in Eq. [4] [21].

$$Qt = k_0 t \quad [4]$$

First order equation shows that release drug depends upon its concentration Eq. [5] [22]:

$$Log\ Qt = LogQ_0\_K1t \quad [5]$$

Higuchi Model is described by Eq. [6] [23]:

$$Qt = KHt^{1/2} \quad [6]$$

Hixon-Crowell equation is shown in Eq. [7] [24]:

$$Q_0^{1/3} - Qt^{1/3} = K_{HC}t \quad [7]$$

Korsmeyer-Peppas Model is shown by Eq. [8] [25]:

$$\frac{M_t}{M_0} = K_{KP}n \quad [8]$$

where K_0, K_1, K_H, K_{HC}, and K_{KP} are the rate constants, respectively, and Mt is the amount of drug release at time t; M_0 is the amount of drug release at time ∞ and n = release exponent, if 0.5 < n < 1.0 it is a non Fickian release and n = 1 is a case of II transport.

RESULTS AND DISCUSSION

Solubility studies

The solubility of 5-FU was found to be 0.118 at pH 1.2, 0.11 at pH 6.8, and 0.10 at pH 7.4, which indicates a considerable influence on solubility of drug. It was observed that increasing the concentration of HP-β-CD results in a linear enhancement in solubility of 5-FU.

Scanning electron microscopy

The SEM photomicrographs revealed discrete spherical microbeads formation with slightly rough surfaces having a presence of drug crystals **Fig. 2A** [26]. The average diameter of microbeads containing 5-Fluorouracil ranges up to 10 um. This is attributed due to increase in the polymeric concentration that results in large size microbeads. These microbeads were held by rigid polymeric network [27]. The surface structure contains numerous drug particles scattered throughout the surface of microbeads with aggregation [28]. However empty microbeads represent their small size with smooth surface as shown in **Fig. 2B**.

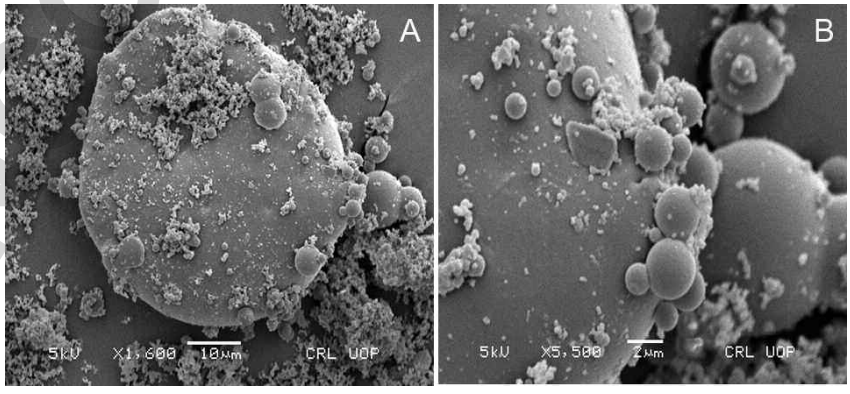


Figure 2. SEM of drug loaded microbeads (A) and empty microbeads (B).

X-ray diffraction (XRD)

XRD analysis was carried out in order to investigate drug polymorphism after being entrapped. XRD of pure drug, empty microbeads and drug loaded microbeads is displayed in Figure 3 (A, B & C respectively). Intense diffraction peaks are observed at 2θ =18.9°, 20.6°, 28.5° in the diffraction pattern of pure 5-Fluorouracil, indicating its crystalline state. The microbeads containing drug shows the presence of all peaks appeared at same 2θ values as observed in empty microbeads but with much less intensity. The absence of peak at 2θ of 28° indicates a molecular dispersion of drug in to polymeric microbeads [29].

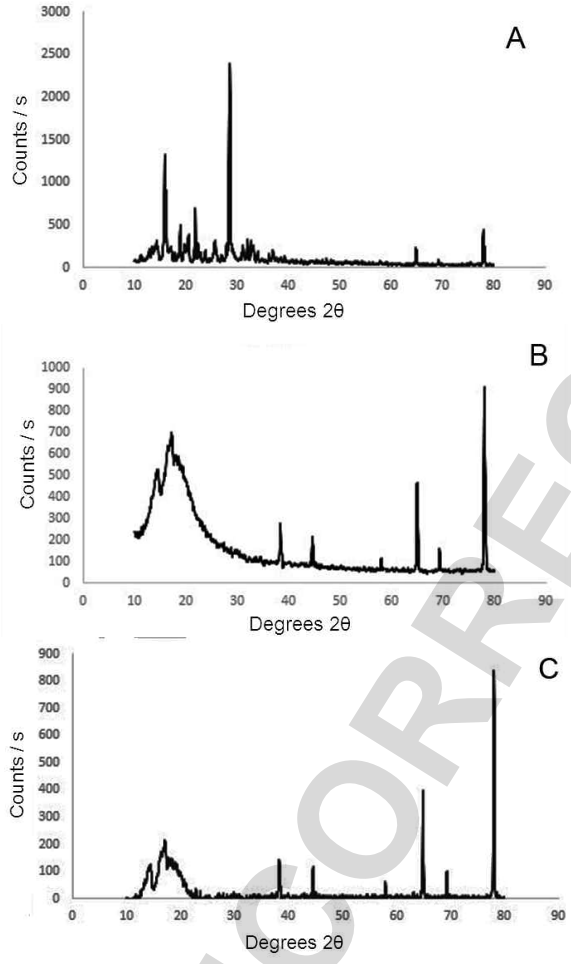


Figure 3. XRD spectra of A) 5-FU, B) empty microbeads, and C) drug loaded microbeads.

Fourier transforms infrared spectroscopy (FTIR)

The FTIR spectra of microbeads containing 5-FU, empty microbeads and drug containing microbeads as shown in Fig. 4 was taken to investigate incompatibility between 5-FU and polymer used. The 5-FU shows a characteristic peak at 3124.05cm^{-1} due to N-H stretching. The peak at 1720.81cm^{-1} and 1645.07 is due to C=O stretching. The appearance of peak at 1242.90 cm^{-1} and 803.35 cm^{-1} shows CH in plane and CH out of plane. The presence of pyrimidine ring [30] is confirmed by banned appeared at 747.56 cm^{-1}.

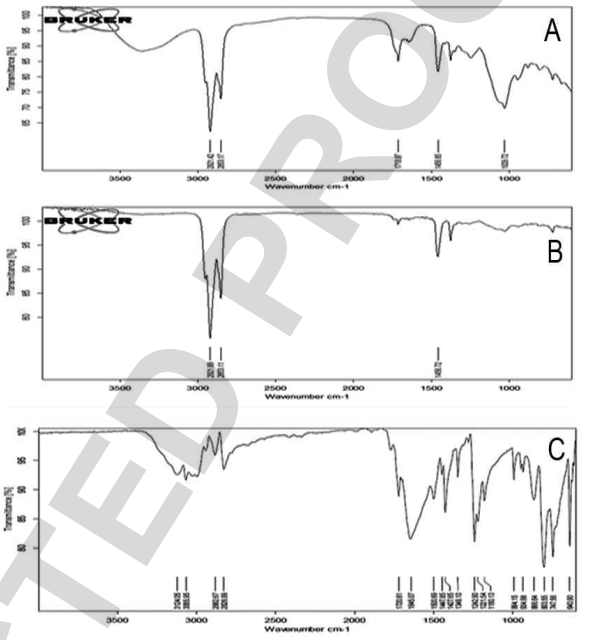


Figure 4. FTIR spectra of A) drug loaded microbeads, B) empty microbeads, and C) 5-FU.

FTIR spectra of microbeads containing 5-FU and empty microbeads shows no evidence of appearance and disappearance of represented peaks, indicating absence of any chemical interaction between formulation components [31].

Differential scanning calorimetry (DSC/TGA)

DSC was performed to understand the thermal behavior of pure drug 5-FU, HP-β-CD, microbeads containing drug and empty microbeads as presented in **Fig. 5**. In case of 5-FU a sharp peak at 289 °C indicates its melting point. The microbeads containing 5-FU shows the absence of endothermic as the drug is molecularly dispersed in the polymeric microbeads showing amorphous nature of drug [32].

Thermal properties of polymers can be confirmed by TGA. TGA tracing shows the weight loss occurs with respect to increase temperature for pure polymers. TGA pattern of empty microbeads and microbeads containing drug show almost same behavior

because of their similar chemical nature. The initial weight loss at 100 °C indicates loss of moisture and second loss after 250 °C indicates the degradation of polymers. However 28% weight loss in 5-FU occurred at 300 °C corresponding to TGA curve.

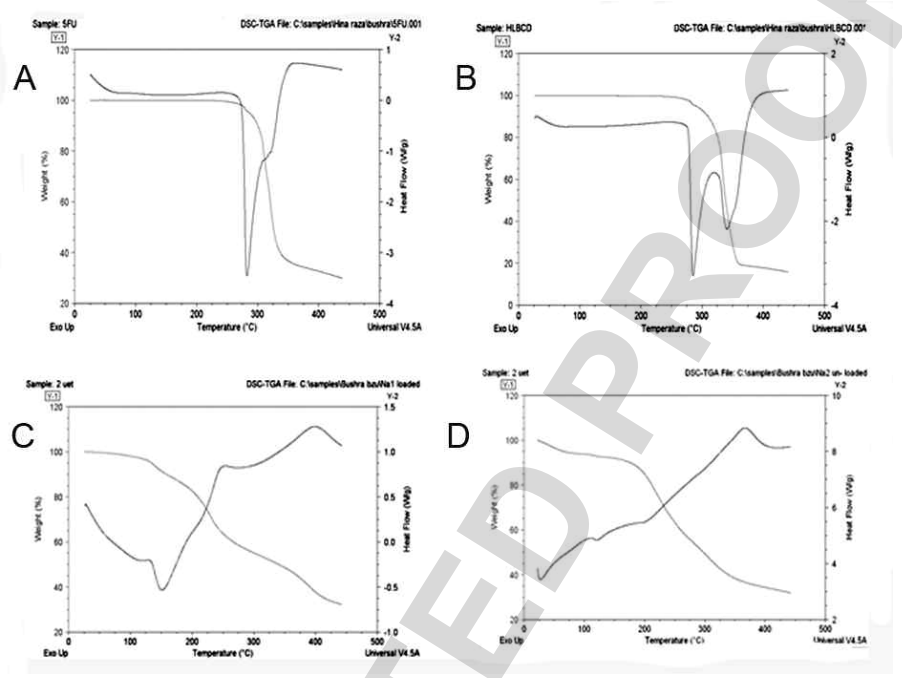


Figure 5. DSC Spectrum of A) 5-fluorouracil, B) hydroxypropyl-β-cyclodextrin, C) drug loaded microbeads, and D) empty microbeads.

Test for glutaraldehyde residue

The formulated microbeads were tested for the residue of glutaraldehyde by gas chromatography. The result shows that all formulation of prepared microbeads was found to be free from residual glutaraldehyde as shown in **Fig. 6**. The peak observed at 16.0 were shown in both figures represents glutaraldehyde concentration in pure form (**Figure A**). However, more concentration of glutaraldehyde in **Figure A** was observed *i.e.* 10 which was reduce upto 1.25 in **Fig. 6B** which indicates that microbeads were free from toxic limit of glutaraldehyde.

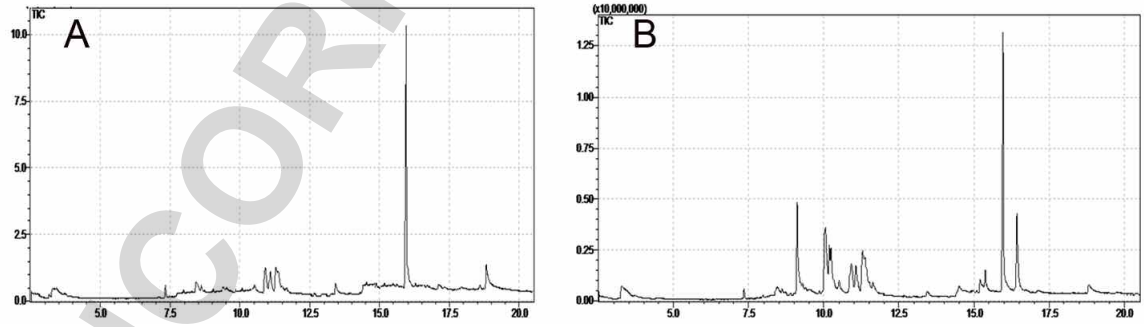


Figure 6. Gas chromatogram of A) pure glutaraldehyde and B) sample microbeads.

Swelling studies

The drug containing microbeads exhibited higher degree of swelling, compare to empty microbeads. These findings suggest that microbeads swell effectively in aqueous environment due to hydration. Following hydration pores or channels were formed in the microbeads exhibiting marked degree of swelling when exposed to pH 7.4 [33]. These findings suggest effective swelling at pH 7.4.

Drug loading and entrapment efficiency

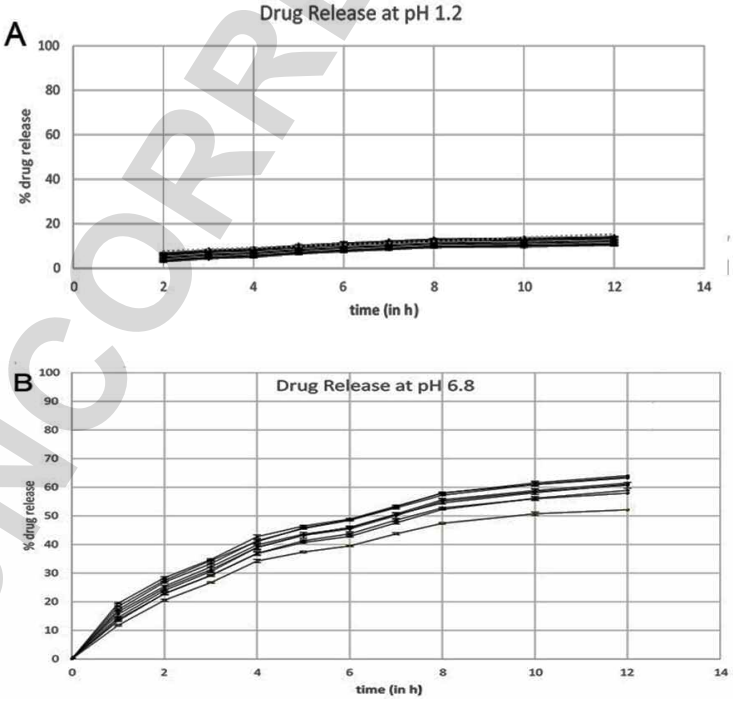
The encapsulation efficiency of prepared microbeads formulation NB1 to NB9 was 31.37%, 32.33%, 32.83%, 37.55%, 39.86%, 39.32%, 42.17%, 45.22%, and 45.61%, respectively, as shown in Table 2. It was observed that encapsulation efficiency shows a significant increase by increasing concentration of polymer. It is believed as the concentration of polymer increases, viscosity of dispersed phase also increases that restrict the mobility of drug inside the droplet with enhanced encapsulation efficiency [34].

Formulation code	Encapsulation efficiency	Percent drug loading	% water uptake pH 7.4
NB1	31.35 ± 0.020	62.70 ± 0.041	10%
NB2	32.25 ± 0.130	64.50 ± 0.260	10%
NB3	32.75 ± 0.127	65.52 ± 0.251	10%
NB4	35.29 ± 0.228	70.59 ± 0.457	15%
NB5	39.82 ± 0.182	79.65 ± 0.369	15%
NB6	39.26 ± 0.106	78.52 ± 0.213	15%
NB7	41.70 ± 0.066	83.40 ± 0.133	20%
NB8	45.23 ± 0.076	90.47 ± 0.152	20%
NB9	45.70 ± 0.086	91.41 ± 0.173	20%

Table 2. Encapsulation efficiency, percent drug loading and degree of swelling of 5-Fluorouracil loaded microbeads.

In vitro drug release

The release pattern of formulated microbeads appears to be increased by using the microbeads having higher concentration of 5-FU as compare to microbeads of low drug concentration as shown in the **Fig. 7** from NB1 to NB9. *In vitro* drug release was carried out in three different physiological pH buffer solutions (pH 1.2, 6.8, and 7.4).



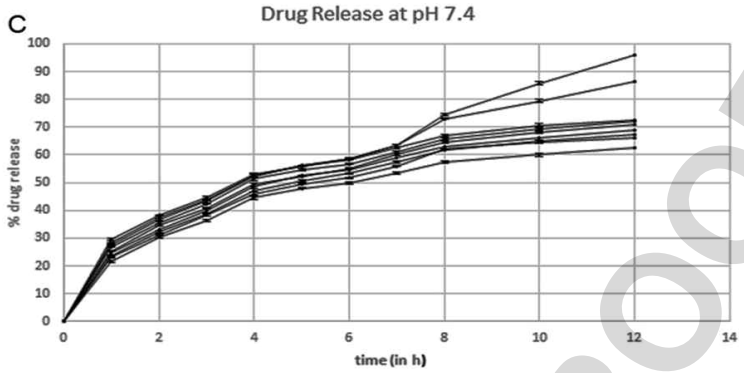


Figure 7. Percentage release of 5-FU from microbeads at pH 1.2 (A), at pH 6.8 (B), and at pH 7.4 (C).

All prepared formulation from NB1 to NB9 shows a minimum amount of drug release at pH 1.2 which is less than 20 % due to pH dependency of the sodium alginate. A sudden increase in release rate was observed at pH 6.8 which was up to 60 % due to hydrolysis of sodium alginate above than its pKa value 4.8 and maximum drug release was observed at pH 7.4 which was above 80% due to combine effect of HP-β-CD and sodium alginate. At pH 7.4 water from surrounding environment penetrates into microbeads as a consequence, the microbeads turn into hydrogels and have their diameter increased, favoring the drug diffusion. Furthermore, the smaller size microbeads formed at low polymeric concentration have greater surface area exposed to dissolution medium, cause increase in drug release.

Drug release kinetics

Different kinetic release models were applied. The observed value of R^2 of zero order release was found to be from 0.89 to 0.96 and value of first order release was from 0.92 to 0.98. The value of R^2 of Higuchi was from 0.98 to 0.99 and 0.95 to 0.97 for Hixon-Crowell. It was concluded that the prepared formulations follows first order release and Higuchi model suggesting drug release followed a diffusion process. The mechanism of drug release was best studied using Korsmeyer-Peppas model and value of n was found to be 0.6 which was < 0.7 showing a Fickian drug release mechanism as shown in **Table 3.**

Sr. #	**Zero Order**		**First Order**		**Higuchi Model**		**Korsmeyer-Peppas**			**Hixon-Crowell**	
	pH 1.2										
	R^2	K_O	R^2	K_1	R^2	K_H	R^2	K_{HP}	n	R^2	K_H
NB1	0.9327	1.419	0.9412	0.015	0.9942	4.068	0.9953	0.438	0.455	0.9384	0.005
NB2	0.9215	1.513	0.9311	0.016	0.9925	4.356	0.9958	5.081	0.420	0.9280	0.005
NB3	0.9266	1.471	0.9357	0.016	0.9934	4.226	0.9956	4.788	0.435	0.9328	0.005
NB4	0.9447	1.312	0.9526	0.014	0.9945	3.739	0.9945	3.727	0.502	0.9496	0.005
NB5	0.9507	1.253	0.9575	0.013	0.9937	3.561	0.9939	3.353	0.531	0.9553	0.004
NB6	0.9173	1.549	0.9273	0.017	0.9915	4.463	0.9959	5.324	0.408	0.9240	0.005
NB7	0.9643	1.099	0.9694	0.012	0.9874	3.091	0.9915	2.426	0.626	0.9678	0.004
NB8	0.9605	1.147	0.9661	0.012	0.9901	3.237	0.9924	2.701	0.593	0.9643	0.004
NB9	0.9668	1.064	0.9716	0.011	0.9850	2.984	0.9906	2.222	0.651	0.9700	0.004

	pH 6.8										
	R^2	K_O	R^2	K_1	R^2	K_H	R^2	K_{HP}	n	R^2	K_H
NB1	0.9384	5.608	0.9767	0.081	0.9918	15.980	0.9917	15.488	0.516	0.9668	0.024
NB2	0.9417	6.208	0.9829	0.094	0.9933	17.682	0.9932	17.086	0.518	0.9731	0.027
NB3	0.9400	6.493	0.9832	0.101	0.9947	18.527	0.9947	18.500	0.501	0.9731	0.029
NB4	0.9477	6.194	0.9853	0.093	0.9949	17.613	0.9949	16.662	0.529	0.9764	0.027
NB5	0.9362	6.519	0.9809	0.102	0.9953	18.639	0.9956	19.271	0.483	0.9703	0.029
NB6	0.9435	6.536	0.9858	0.101	0.9938	18.608	0.9937	17.873	0.521	0.9762	0.029
NB7	0.9292	6.916	0.9794	0.113	0.9950	19.837	0.9964	21.624	0.455	0.9678	0.032
NB8	0.9348	6.813	0.9827	0.109	0.9948	19.484	0.9952	20.197	0.481	0.9718	0.031
NB9	0.9330	6.856	0.9817	0.111	0.9953	19.632	0.9959	20.802	0.470	0.9706	0.032
	pH 7.4										
	R^2	K_O	R^2	K_1	R^2	K_H	R^2	K_{HP}	n	R^2	K_H
NB1	0.9075	6.910	0.9668	0.114	0.9900	19.952	0.9957	23.961	0.405	0.9522	0.033
NB2	0.9117	7.412	0.9736	0.129	0.9915	21.384	0.9965	25.468	0.409	0.9595	0.036
NB3	0.9132	7.768	0.9761	0.140	0.9923	22.413	0.9976	26.849	0.405	0.9626	0.039
NB4	0.9144	7.296	0.9737	0.125	0.9915	21.027	0.9957	24.689	0.416	0.9603	0.035
NB5	0.9054	7.933	0.9738	0.147	0.9899	22.935	0.9971	28.323	0.390	0.9593	0.041
NB6	0.9080	7.597	0.9735	0.135	0.9903	21.937	0.9962	26.516	0.401	0.9589	0.038
NB7	0.9439	8.804	0.9816	0.172	0.9964	25.192	0.9970	27.398	0.456	0.9764	0.047
NB8	0.9673	9.201	0.9779	0.179	0.9926	26.045	0.9938	23.819	0.546	0.9792	0.049
NB9	0.8976	8.094	6.9717	0.154	0.9875	23.447	0.9971	29.802	0.375	0.9561	0.042

Table 3. In vitro model dependent kinetic studies of 5-FU microbeads.

Statistical analysis

Two-way ANOVA were successfully applied and the p values less than 0.05 and the interaction were found to be significant.

CONCLUSION

Hydroxypropyl-β-cyclodextrin and sodium alginate microbeads were successfully prepared using a simple cross linking method. These microbeads shows a good potential for producing sustained release effect using anticancer drug 5-Fluorouracil and could sustained the drug release up to 12 h.

REFERENCES

1. Işiklan, N. (2006) *J. Appl. Polym. Sci.* **99**: 1310-9.
2. Vaithiyalingam, S., M. Nutan, I. Reddy & M. Khan (2002) *J. Pharm. Sci.* **91**: 1512-22.
3. Uhrich, K.E., S.M. Cannizzaro, R.S. Langer & K.M. Shakesheff (1999) *Chem. Rev.* **99**: 3181-98.
4. Menon, T.V. & C. Sajeeth (2013) *Res. J. Pharm. Technol.* **6**: 392-7.
5. Costa, P. & J.M.S. Lobo (2001) *Eur. J. Pharm. Sci.* **13**: 123-33.
6. Winzenburg, G., C. Schmidt, S. Fuchs & T.

Kissel (2004) *Adv. Drug Deliv. Rev.* **56**: 1453-66.
7. Saboktakin, M.R., A. Maharramov & M.A. Ramazanov (2009) *Carbohyd. Polym.* **77**: 634-8.
8. Krauland, A.H. & M.J. Alonso (2007) *Int. J. Pharm.* **340**: 134-42.
9. Bradford, M.M. (1976) *Anal. Biochem.* **72**: 248-54.
10. Maestrelli, F., M. Garcia-Fuentes, P. Mura & M.J. Alonso (2006) *Eur. J. Pharm. Biopharm.* **63**: 79-86.
11. Chan, L. & P. Heng (1998) *J. Microencapsul.* **15**: 409-20.
12. Pluemsab, W., Y. Fukazawa, T. Furuike, Y. Nodasaka & N. Sakairi (2007) *Bioresource Technol.* **98**: 2076-81.
13. Barbato, F., B. Cappello, M.I. La Rotonda, A. Miro & F. Quaglia (2003) *J. Incl. Phenom. Macro. Chem.* **46**: 179-85.
14. Iliescu, T., M. Baia & V. Miclăuş (2004) *Eur. J. Pharm. Sci.* **22**: 487-95.
15. Lahiani-Skiba, M., Y. Boulet, I. Youm, F. Bounoure, P. Vérité, P. Arnaud, *et al.* (2007) *J. Incl. Phenom. Macro. Chem.* **57**: 211-7.
16. Miro, A., A. Rondinone, A. Nappi, F. Ungaro, F. Quaglia & M.I. La Rotonda (2009) *Eur. J. Pharm. Biopharm.* **72**: 76-82.
17. Arunkumar, P.D. & M. Saravanan (2013) *J. Appl. Pharm. Sci.* **3**: 17-22.
18. Connors, K.A. (1995) *J. Pharm. Sci.* **84**: 843-8.
19. Ganguly, K., T.M. Aminabhavi & A.R. Kulkarni (2011) *Indust. Engin. Chem. Res.* **50**: 11797-807.
20. Xin, J., Z. Guo, X. Chen, W. Jiang, J. Li & M. Li (2010) *Int. J. Pharm.* **386**: 221-8.
21. Fertah, M., A. Belfkira, M. Taourirte & F. Brouillette (2015) *BioResources* **10**: 5932-48.
22. Desai, S.J., P. Singh, A.P. Simonelli & W.I. Higuchi (1966) *J. Pharm. Sci.* **55**: 1230-4.
23. Gupta, P.K., C. Hung & D. Perrier (1986) *Int. J. Pharm.* **33**: 137-46.
24. Ali, J., S. Arora, A. Ahuja, A.K. Babbar, R.K. Sharma & R.K. Khar (2007) *AAPS PharmSciTech.* **8**: 312-9.
25. Kamble, R., M. Maheshwari, A. Paradkar & S. Kadam (2004) *AAPS PharmSciTech.* **5**: 75-83.
26. Rahman, Z., K. Kohli, R.K. Khar, M. Ali, N.A. Charoo & A.A. Shamsher (2006) *AAPS PharmSciTech.* **7**: E113-21.
27. Banerjee, S., G. Chaurasia, D. Pal, A.K. Ghosh, A. Ghosh & S. Kaity (2010) *J. Sci. Indust. Res.* **69**: 777-84.
28. Parikh, R.H., J.R. Parikh, R.R. Dubey, H.N. Soni & K.N. Kapadia (2003) *AAPS PharmSciTech.* **4**: 14-21.
29. Li, P., Y. Wang, Z. Peng, F. She & L. Kong (2011) *Carbohyd. Polym.* **85**: 698-704.
30. Olukman, M. & E.K. Solak (2012) *J. Biomat. Nanobiotech.* **3**:469.
31. Raffin, R., L. Colomé, A. Pohlmann & S. Guterres (2006) *Eur. J. Pharm. Biopharm.* **63**: 198-204.
32. Mu, L. & S. Feng (2001) *J. Control. Release* **76**: 239-54.
33. Martinac, A., J. Filipović-Grčić, D. Voinovich, B. Perissutti & E. Franceschinis (2005) *Int. J. Pharm.* **291**: 69-77.
34. Kumar, B.P., I.S. Chandiran, B. Bhavya & M. Sindhuri (2011) *Indian J. Pharm. Sci. Res.* **1**: 19-37.
35. Dey, S., S. Pramanik & A. Malgope (2011) *ISRN Pharm.* **2011**: 627623
36. Boppana, R., R.V. Kulkarni, S.S. Mutalik, C.M. Setty & B. Sa (2010) *J. Microencapsul.* **27**: 337-44.