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An in vitro release study of 5-fluoro-uracil (5-FU) from swellable poly-(2-hydroxyethyl methacrylate) (PHEMA) nanoparticles

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Abstract Nanomaterials are at the leading edge of the rapidly developing field of nanotechnology. The use of nanoparticles as drug delivery vehicles for anticancer therapeutics has great potential to revolutionize the future of cancer therapy. The present paper concerns both the optimizations of anticancer drug loading and its release from polymeric nanoparticles. The major aim of this study was to design poly (HEMA) nanoparticles as swelling controlled drug release system for anticancer drug. The prepared nanoparticles were characterized by Infra-Red (IR) Spectra, Particle size Analysis, and Scanning Electron Microscopy (SEM). The nanoparticles were loaded with widely used anticancer drug, 5-Fluorouracil, and controlled release of drug was investigated to observe the effects of various parameters such as percent loading of the drug, chemical architecture of the nanocarriers, pH, temperature, and nature of release media on the release profiles. The chemical stability of 5-Fluorouracil (5-FU) was also tested in phosphate buffer saline (PBS) (pH = 7.4) and release was studied in various simulated biological fluids. The prepared nanoparticles could provide a possible pathway for controlled and targeted delivery of anticancer drug, thus causing lower side effects and higher efficacy.

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1 Introduction

The aim of any anticancer research is to improve patient survival after chemo- or radiotherapy. Radiotherapy is utilized in specific type of tumours while chemotherapy is very common for a variety of cancers [1]. Unfortunately, traditional anticancer therapy results by a lot of side effects that involve damage of healthy cells leading to the miserable life for cancer patients. So, the effectiveness of a treatment is related to the development of multifunctional delivery systems, which can target subtle molecular alterations that distinguish a transformed cell from the healthy cells found in the body [2]. Chemotherapy is a general term used to describe various drugs to treat cancer that work throughout the body. These drugs can be administered individually or can be combined (combination chemotherapy). Chemotherapy drugs are designed to interfere with or kill fast growing cancer cells and affect other fast growing cells in the body. For cancer, regional or local administration is possible for certain lesions such as head and neck cancer and melanoma. For deep-seated tumours, limited targeting has been achieved via selective delivery using upstream intraarterial administration of microspheres [3], use of immunoliposomes [4], mostly at the preclinical stage.

Drug delivery systems have already had an enormous impact on medical technology, greatly improving the performance of many existing drugs and enabling the use of entirely new therapies [5]. These delivery systems offer numerous advantages compared to conventional dosage forms, which include improved efficacy, reduced toxicity, and improved patient compliance and convenience. In controlled drug delivery, drug release generally occurs by one of the three main mechanisms: (1) Diffusion, (2) Chemical Reaction, and (3) Solvent activation and

transport [6]. Controlled drug delivery applications include both (over days/Weeks/Months/Years) delivery and targeted (e.g. to a tumour, diseased vessel, etc.) delivery on a one time or sustained basis [7]. Targeting delivery of drug to the diseased lesions is one of the important aspects of drug delivery systems (DDS). To convey a sufficient dose of drug to the lesion suitable carrier of drug to the lesion are needed [8]. Most of the new drug delivery systems and the final dosage form incorporating them require drug substances in a particulate form with specific biopharmaceutical, physiochemical, and size properties. Smaller particles have larger surface area; therefore, most of the drug associated could be at or near the particle surface leading to fast drug release whereas, larger particles have large cores which allow more drug to be encapsulated and diffuse out [9]. Thus, the ultimate goal of a drug delivery system is to deploy medication intact and to specifically target sites through a medium that can control the drug administration by either physiological or chemical triggers.

Polymeric nanoparticles present a higher stability, when in contact with biological fluids and their polymeric nature allows obtaining the desired controlled and sustained release. The advantage that nanoparticles hold over other drug delivery systems is their submicron size which makes extravasations possible and occlusion of terminal blood vessel unlikely [10]. Nanoparticles exhibit attractive physiochemical properties like particle size, high stability, lower toxicity, targeted drug delivery hydrophilic–hydrophobic balance and ability to modify their surface characteristics easily [11] and for this reason they have been recognized as potential drug carriers for bioactive ingredients such as anticancer drugs [12], vaccines [13], and oligonucleotides [14].

Nanoparticles are excellent tumour targeting vehicles because of a unique inherent property of solid tumours. Due to rapid growth of solid tumors, many tumors present with fenestrated vasculature and poor lymphatic drainage, resulting in an enhanced permeability and retention (EPR) effect [15] which allows nanoparticles to accumulate specifically at the tumor site. Although nanoparticles protect the drug from rapid metabolism and clearance, as well as non-specific recognition and distribution, stealth shielding nanoparticles [16], in addition, will help to avoid uptake by the reticuloendothelial system [17] and mononuclear phagocytes [18].

An exhaustive work has been done in the field of controlled drug delivery of antitumor drugs through polymeric nanocarriers. The proposed study could prove to be a potential method of delivery of antitumor drugs to the targeted sites using PHEMA nanoparticles. PHEMA is particularly attractive for biomedical engineering applications because its physical properties can be easily manipulated through formulation chemistry and it has been extensively used in medical applications such as contact lenses [12], keratoprotheses, and as orbital implants [19]. Thus, in the present paper, we are reporting results on swelling controlled release of antitumor drug, from the drug loaded PHEMA nanoparticles. The drug chosen for the study is 5-Fluorouracil (5-FU) that belongs to a family of antitumor drugs. It is one of the oldest antitumor drugs, commonly used in clinical oncology practice. It is widely used in clinical treatment of several solid cancers such as gastrointestinal, pancreas, breast, colorectal, liver and brain cancer [20]. The target sites of 5-FU are all the organs of the human body [21], specially the gastrointestinal tract. 5-FU is quickly metabolized in the body; therefore, the maintenance of high serum concentrations of this drug is needed to improve its therapeutic activity. The maintenance of these serum concentrations requires continuous administration but 5-FU shows severe toxic effects; and of course reaching and/or exceeding the toxic concentrations must be avoided [22]. The delivery of this drug by PHEMA nanoparticles offers the possibility of maximizing its efficacy and safety and provides a suitable rate of delivery of the therapeutic dose, at the most appropriate site in the body, in order to prolong the duration of pharmacological activity, to reduce the side effects and to minimize the administration frequency thus enhancing patient compliance [23].

2 Experimental

2-Hydroxyethyl methacrylate (HEMA, Sigma Aldrich Co.) and ethyleneglycol dimethacrylate (EGDMA, Sigma Aldrich Co.) were used as monomer and cross-linking agent, respectively. Benzoyl peroxide (BPO, MERCK) and polyvinyl alcohol (Mol. Wt. 14000) (PVA, MERCK) were used as the initiator and the stabilizer, respectively. Toluene (MERCK) was selected as the diluent. All chemicals were of analytical grade and doubly distilled water was used throughout the experiments.

2.1 Methodology

2.1.1 Purification of monomer

Because of poor stability of HEMA, high purity of the monomer is essentially required in hydrogels synthesis as the presence of impurities may greatly affect the swelling characteristics of the end polymer. Degradation of monomer during transportation and storage at ambient temperatures may result in increased levels of methacrylic acid (MAA) and the natural occurring cross-linker EGDMA. As illustrated in Fig. 1, the HEMA monomer readily undergoes three common reactions:

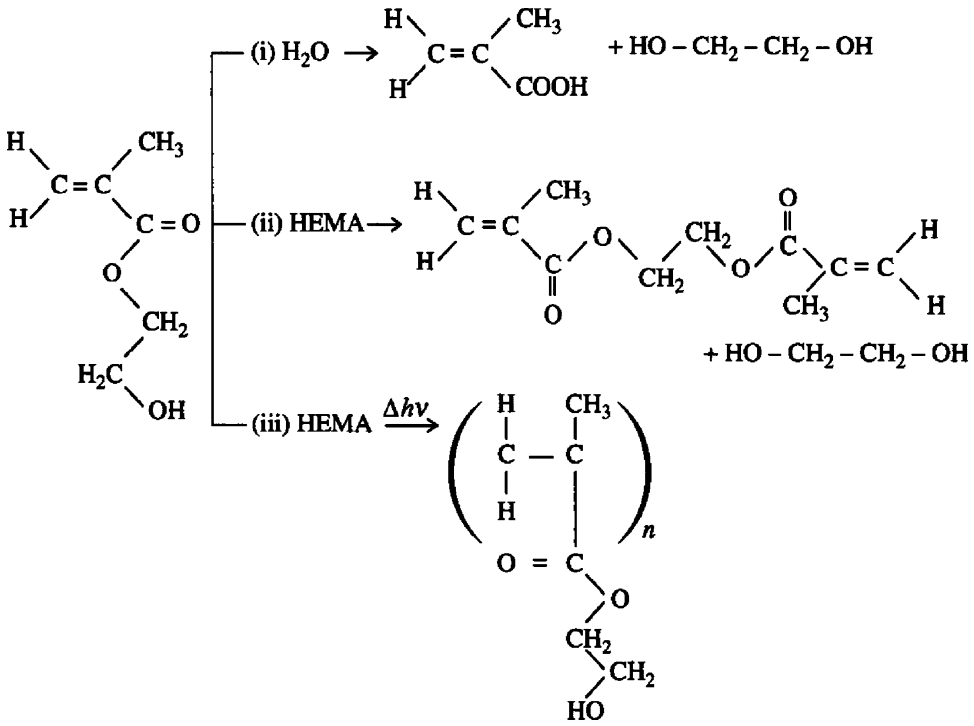

Fig. 1 Synthesis of monomer HEMA

(1) HEMA may hydrolyze at the ester linkage to form MAA and ethylene glycol; (2) two molecules of HEMA may transesterify to form the cross linker and ethylene glycol; (3) monomer may polymerize at the double bond resulting in oligomer and polymer. Although an inhibitor such as hydroquinone (300 ppm) is normally added to minimize the later reactions, an ultra purity is desired for reliable experimental data.

The impurity of MAA in HEMA monomer was removed by stirring the monomer with 15% by weight of anhydrous sodium carbonate for 3 h at 24°C, then vacuum filtering through Whatman filter papers. The yield on an initial volume of 100 ml of HEMA was 89%.

The impurity of EGDMA was then removed by first dissolving the above treated monomer in three times its volume of distilled water. Four extractions were performed with 50 ml of 1:1 (volume) mixture of carbon-tetrachloride and cyclohexane, allowing the layers to separate for 30 min between two extractions. The organic layer containing EGDMA was discarded after each extraction and the aqueous phase was placed under vacuum to remove any remaining organic solvent. The HEMA was then salted out with 100 g of NaCl, then dried with anhydrous sodium sulphate, and filtered.

The partially purified HEMA monomer was vacuum distilled in the presence of 1 g of hydroquinone (added to prevent polymerization) at 60 mmHg and after distillation; the pure HEMA was transferred to an opaque glass bottle and stored at 0°C until use.

2.1.2 Purity of HEMA

The purity of distilled HEMA was determined by high-pressure liquid chromatography (HPLC), [Backmen System (Gold 127)] equipped with a ultraviolet detector, a 25 cm × 46 mm id separation columns ODS (C_{18}), 5 μm particle size. The UV detector was set at 217 nm. The mobile phase was methanol-water (60:40 v/v) and the flow-rate was kept at 1 ml/min. All samples were diluted with pure methanol to 1/1600. 10 μl samples were injected for each analysis. Samples of known concentrations of MAA and EGDMA were injected into the HPLC and the resultant chromatogram was used to construct a standard curve of known concentrations vs. area under the curve. The chromatograph showed three distinct peaks. The first peak, at 3.614 min was identified as MAA. The next peak at 5.503 min was the major peak due to HEMA monomer. The amounts of impurities of MAA and EGDMA in the monomer samples were found to be less than 0.01 mol% MAA and 0.001 mol% EGDMA.

2.1.3 Preparation of nanoparticles

For pharmaceutical use preparative methods of nanoparticles are broadly divided into two categories, those based on physiochemical properties such as phase separation and solvent evaporation [24], and chemical reactions such as polymerization and polycondensation. In the present study cross-linked PHEMA nanoparticles were prepared by a

modified suspension polymerization technique, as published by Kiparissides et al. [25]. Briefly, the method is described as below.

In this technique, polymerization was carried out in an aqueous phase containing PVA, which was used as the stabilizing agent. The mixture containing the monomer HEMA, the cross-linker EGDMA and the initiator Bz_2O_2 dispersed in toluene was added into 500 ml conical flask containing the suspension medium (200 ml aqueous PVA solution (0.5% W/V)). The reactor was flushed by bubbling nitrogen and then sealed. The reaction mixture was placed on magnetic stirrer and heated by vigorous stirring (600–700 rpm) at 80°C for 2 h and then at 90°C for 1 h. The cross-linking reaction was completed within 3 h. After cooling the polymeric particles were separated from the polymerization medium by washing thrice with toluene and twice with acetone. The collected nanoparticles were dried at room temperature to obtain as fine white powder and thereafter stored in airtight polyethylene bag.

2.1.4 Characterization of PHEMA nanoparticles

Nanoparticles of varying compositions were produced by varying preparation conditions and characterized at two levels—the first one is physicochemical characterization, and the other one is biopharmaceutical characterization.

2.1.4.1 Physicochemical characterization Under physicochemical characterization particles were characterized by the following methods:

FTIR spectral analysis: IR spectral analysis was used for structural characterization of nanoparticles. The IR spectra of cross-linked PHEMA nanoparticles were recorded on a FTIR spectrophotometer (Perkin-Elmer, 1000 Paragon) (Shimadzu). While recording FTIR spectra KBr disc method was used for preparation of samples.

SEM (Scanning Electron Microscopy) analysis: Morphological studies of cross-linked PHEMA nanoparticles were performed on scanning electron micrographs (SEM) operated at 10 kV. SEM observations were carried out after gold sputtering the samples with a Philips, 515, fine coater.

Particle size analysis: Nanoparticles were characterized by particle size analysis for size and size distribution. The particle size analysis of prepared nanoparticles was performed on a particle size analyzer (Malvern Mastersizer 2000).

Surface potential (zeta potential) measurements: The zeta potential of a nanoparticle is commonly used to characterize the surface charge property of nanoparticles [26]. It reflects the electrical potential of particles and is influenced by the composition of the particles and the medium in which they are dispersed. The zeta potential can also be used to determine whether a charged active material is encapsulated within the centre of the nanocapsule or adsorbed onto the surface. In order to understand the nature of the drug 5-FU-nanoparticle interaction zeta potential studies were performed with a digital potentiometer (Model No. 118, EI Product, Mumbai, India).

2.1.4.2 Biopharmaceutical characterization Swelling studies in nanoparticles: A fundamental relationship exists between the swelling of a polymer in a solvent and the nature of the polymer and the solvent. The swelling properties of hydrogels affect their usability as a biomaterial in medicine pharmacy and veterinary practice [27].

Swelling of nanoparticles was studied by a conventional gravimetric procedure [28]. In a typical experiment 0.1 g of nanoparticles were allowed to swell in a definite volume (10 ml) of PBS taken in a preweighed sintered glass crucible (pore size 5–10 μm) and weighed after a definite period by removing excess PBS by vaccum filtration. The swelling process of nanoparticles was monitored continuously up to 15 min after which no weight gain of swollen nanoparticles was recorded which clearly indicates equilibrium swelling condition. The amount of water imbibed by the nanoparticles was calculated from the following equation:

$$\text{Swelling ratio} = \frac{W_t}{W_0} \quad (1)$$

where, W_t is the weight of swollen nanoparticles at time t and W_0 is the weight of dry nanoparticles at time 0.

Loading of drug on to nanoparticles: Ideally, a successful nanoparticulate system should have a high drug loading capacity thereby reducing the quantity of matrix material for administration [29]. Drug loading may be done by two methods: (i) either incorporating at the time of nanoparticles production (encapsulation method), or (ii) absorbing the drug after formation of nanoparticles by incubating the carrier with a concentrated drug solution (adsorption/absorption technique). In the present work, the later method has been adopted as in the former one purification of the loaded device remains a problem.

For loading of nanoparticles known volume of drug 5-FU was taken and diluted with appropriate amount of PBS solution and shaken vigorously for mixing of drug and PBS solution. In brief, drug loaded nanoparticles were prepared by allowing 0.1 g of nanoparticles to swell in freshly prepared drug solution (10 ml) until equilibrium was reached. The % loading of drug onto nanoparticles was calculated by the following equation:

$$\%\ \text{Loading} = \frac{W_d - W_0}{W_0} \times 100 \quad (2)$$

where, W_d and W_0 are the weights of loaded and unloaded nanoparticles, respectively.

2.2 In vitro release experiments

In vitro release of the loaded 5-FU was carried out by placing the dried and loaded nanoparticles (0.1 g) in a test tube containing a definite volume (10 ml) of phosphate buffer saline (PBS) as the release medium (pH = 7.4) (1.2 mM KH_2PO_4, 1.15 mM Na_2HPO_4, 2.7 mM KCl, 1.38 mM NaCl). The resulting suspension was gently shaken for definite time period (3.5 h) and 5 ml supernatant was withdrawn at predetermined time intervals (30 min) from the suspension medium replacing it with fresh PBS. The amount of 5-FU released from the polymeric nanoparticles was measured spectrophotometrically at 265 nm (Shimandzu 1700 Phama Spec.) and the amount of drug released was determined from calibration plot.

2.3 Release kinetics

The drug loaded nanoparticle suspension was taken for 3.5 h to study kinetics of the release process. For monitoring the progress of the release process, 5 ml aliquots were withdrawn at desired time intervals and instantly replaced by fresh release medium (PBS). In the aliquots withdrawn, the amount of drug released was estimated spectrophotometrically.

The kinetic data were analyzed with the help of the following equation, which could be helpful in determining the nature of the release process,

$$\frac{W_t}{W_\infty} = Kt^n \tag{3}$$

where W_t and W_∞ are the amount of the drug release at time t and at infinity time (equilibrium amount of drug released), respectively, and K is rate constant. The exponent n, called diffusional exponent, is an important indicator of the mechanism of drug transport and, in general, has a value between 0.43 and 0.85. The numerical value of n indicates the nature of the release process, i.e. when $n = 0.43$, release is Fickian in nature, when $n = 0.85$, release is of case II type and when n lies between 0 and 0.85, the release process becomes anomalous in nature. For evaluating the diffusion constant of loaded drugs, the following equation can be used:

$$\frac{W_t}{W_\infty} = 4\left(\frac{Dt}{\pi L^2}\right)^{0.5} \tag{4}$$

where, D is the diffusion constant of the drug and L being the diameter of the dry nanoparticles.

2.4 Chemical stability of drug

In order to check the chemical stability of entrapped drug in different release medium, the UV spectral study (Shimandzu 1700 Pharma Spec) was performed [30]. For this purpose UV spectra of pure 5-FU solution and released fractions were compared at different pH and different time periods.

2.5 Statistical analysis

All experiments were done at least thrice and a fair reproducibility was observed. The data summarized in Tables have been expressed as mean ±SD of at least three independent determinations. The plots were drawn taking the mean values and each curve has been shown to include error bars.

3 Results and discussion

3.1 FTIR spectral analysis

The FTIR spectra of pure drug (5-FU) and loaded nanoparticles are shown in Fig. 2(a, b), respectively. The IR spectra (b) of loaded nanoparticles clearly mark the presence of HEMA as evident from the observed bands at 1728 cm^{-1} (C=O stretching), 1172 cm^{-1} (O–C–C stretching), 3556 cm^{-1} (O–H stretching), and 1454 cm^{-1} (O–H bending) respectively. The spectra (b) also mark the presence of drug (5-FU) as evident from the observed bands at 1260 (aromatic C–N stretching) and at 500–600 (aromatic ring region). Similar types of bands are present in spectra (a) of the pure drug (5-FU). Thus the presence of drug in the loaded nanoparticles is confirmed.

3.2 Scanning Electron Microscopy

The SEM image of nanoparticles is shown in Fig. 3, which clearly suggests for a non-smooth morphology of the PHEMA nanoparticles. The SEM image also reveals that the size of nanoparticles is not uniform and varies in the range 100–300 nm.

3.3 Particle size analysis

Particle size and size distribution are one of the most important characteristics of nanoparticle system as they determine biological fate, toxicity and targeting ability of nanoparticle systems, as well as drug loading, drug release and stability of nanoparticles. The interaction of nanoparticles with living systems depends on their characteristic dimensions. Nanoparticles, of a few nm in size, may reach well inside targeted cells, a situation not possible for larger particles. It has been reported that nanoparticles can reach into the blood and other target sites such as liver, heart or blood cells [31].

The particle size distribution curve of prepared nanoparticles is shown in Fig. 4, which implies that the

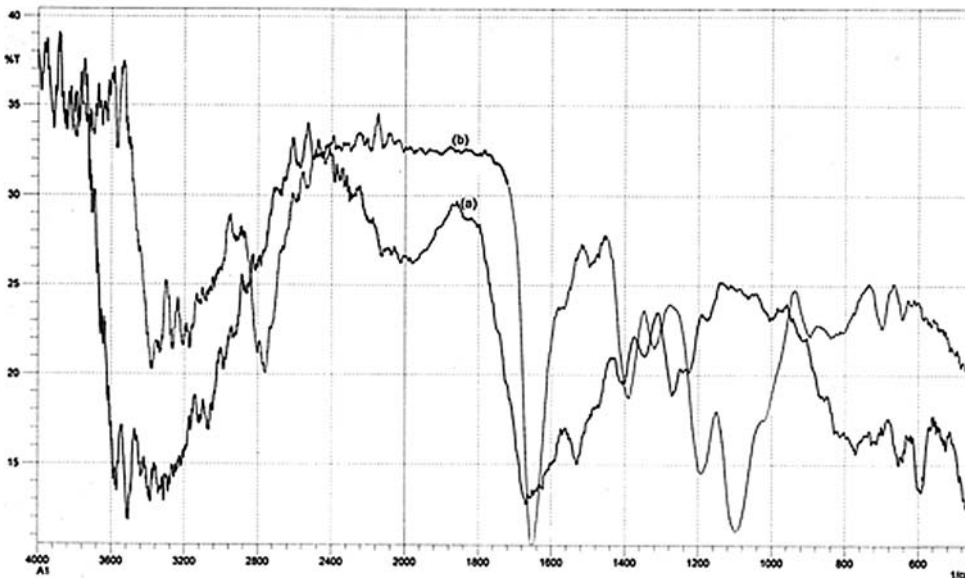

Fig. 2 FTIR spectra of **a** 5-FU and **b** loaded PHEMA nanospheres

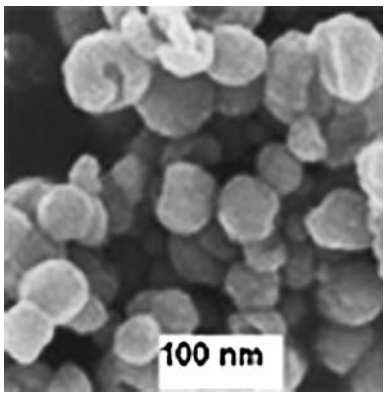

Fig. 3 The SEM images of cross-linked PHEMA nanospheres

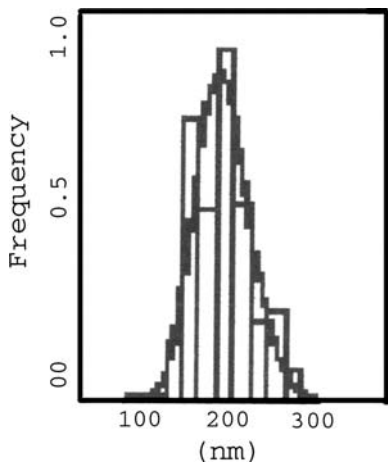

Fig. 4 Particle size distribution curve of cross-linked PHEMA nanospheres

dimensions of nanoparticles vary in the range 100–300 nm which is confirmed by SEM also.

3.4 Surface potential measurements

The zeta potential of a nanoparticle is commonly used to characterize the surface charge property of nanoparticles. The value of ξ potential for unloaded and drug loaded nanoparticles are summarized in Table 1 which clearly indicate that upon loading of 5-FU onto nanoparticle surfaces a net increase in positive potential of the particle surface is noticed. The observed increase is quite obvious and may be explained by the fact that drug molecules bear a positive charge and due to their loading onto the particle surface the positive charge increases on the surface, which clearly provides an evidence of drug surface interaction.

3.5 Mechanism of drug release

A swollen nanoparticle may be imagined as a three dimensional polymer network structure between the strands of which are present water filled permeation channels. The water may be structurally bound to the polymer either as a modified water structure or similar to normal free water in the pure free state. The water occupies the permeation

Table 1 Surface potential of unloaded and loaded PHEMA nanoparticles

pH	EMF (mV) Buffer solution	EMF (mV) Unloaded	EMF (mV) Loaded
1.2	259	350.1	439.7
7.4	−4.5	3.6	10.7
8.6	70.1	−56.3	−15.1

channels when the water-soluble solutes diffuse out to the external receptor medium from within the nanoparticles. A free-volume theory assumes that the free volume of the water present in the hydrogel is available for the diffusion of water-soluble solutes [11].

It is thus clear that when a drug delivery system comes into contact with a solvent, relaxation of polymer chains takes place. This situation arises when the characteristic glassy–rubbery transition temperature of the polymer decreases below the experimental temperature. The dissolved drug passes into the external-receiving medium, crossing the swollen polymeric layer formed around the matrix. The rate of swelling process decides the Fickian or non-Fickian nature of the released drug.

3.6 Release study of 5-Flurouracil

In the present paper the authors have studied the effect of various factors such as concentration of monomer, cross-linker, initiator, and drug on the release rate. The effect of pH, temperature and various physiological fluids on drug release profiles has also been studied. In the present study drug loaded nanoparticles have been designed in such a way that the end product may be used as a solid formulation either as pills or capsules or even in powder form. Thus, we feel that like many other drugs the present nanoparticles may be successfully employed for oral administration.

In the present study, in general the release profiles show a fast release rate followed by a slow delivery of drug into the release medium. This has made the release profiles to look biphasic in nature. The reason for the observed initial fast release could be due to the high initial concentration of drug within the nanoparticle. However, after the initial release is over the remaining amount of drug passes slowly into the release medium because of the fall in concentration of drug within the nanoparticles. This is the reason why biphasic type of release curves are observed in several cases. Moreover, the initial fast release may also be attributed to the burst effect, i.e. accumulation of drug on the surface of the nanoparticles.

3.7 Effect of % loading on 5-FU release

An important aspect in the use of nanoparticles as drug vehicle is the effect of the drug loading level on the drug release rate. Higher drug loading may be achieved either by using highly concentrated drug solution or repeated soaking of nanoparticles in drug solution and then drying them. In the present work, nanoparticles of definite composition were loaded with different amounts of 5-FU by allowing the particles to swell in the drug solution of concentration varying in the range 10 mg/ml to 25 mg/ml. The loaded particles were allowed to release the entrapped drug into a definite volume of release medium. The release results are shown in Fig. 5, which reveal that the released amount of 5-FU increases with increasing percent loading. The observed results may be attributed to the fact in the nanoparticles with higher loading the solvent front (PBS) advances faster into the polymer matrix and, therefore, results in a larger release of the drug. Similar type of results have also been reported by other workers [32].

3.8 Effect of monomer on 5-FU release

Drug release profiles are sensitive to chemical architecture of the carrier as well as the experimental conditions of preparation of drug carrier. The effect of HEMA on the release of 5-FU has been investigated by varying the monomer concentration in the range 12.3–24.7 mM. The release and swelling results are shown in Fig. 6(a, b), which clearly indicate that the cumulative release of 5-FU decreases with increasing concentration of HEMA. The results may be explained by the fact that as the amount of monomer increases in the nanoparticles, the polymeric nanoparticles becomes largely crowded with PHEMA chains and this consequently reduces the free volume accessible to the penetrant water molecules. This obviously brings about a fall in both, the swelling ratio as well as in the released amount of drug.

Another possible reason may be that with increase in PHEMA content the interaction between the polymer chains and drug molecules increase which also results in a lower release of 5-FU.

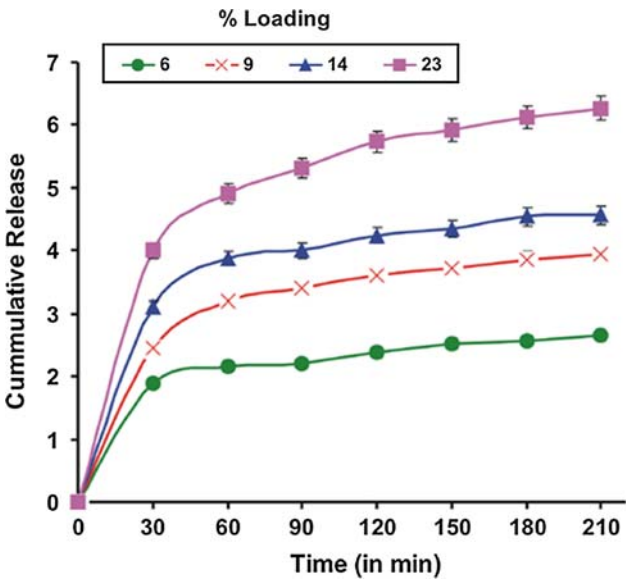

Fig. 5 Effect of % loading of 5-FU on its release profiles from loaded nanospheres of definite composition [HEMA] = 12.37 mM, [EGDMA] = 1.06 mM, [Bz_2O_2] = 0.248 mM, pH = 7.4, temp. = 25°C

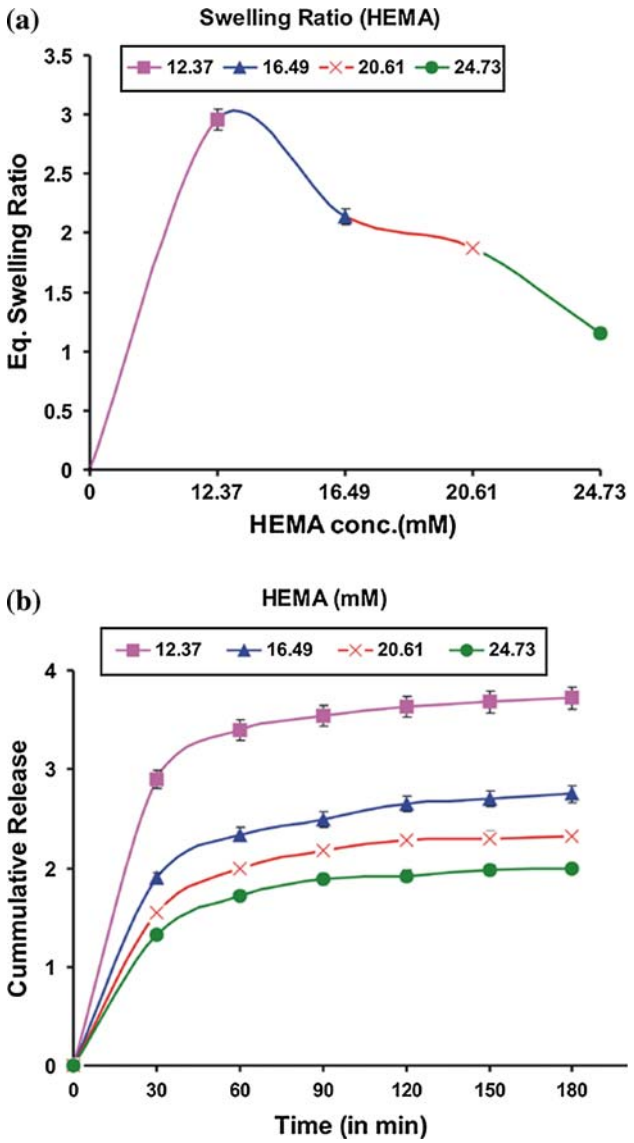

Fig. 6 Effect of monomer [HEMA] content of the nanospheres on the **a** swelling profile and **b** release profile of 5-FU from loaded nanospheres of definite composition [EGDMA] = 1.06 mM, [Bz_2O_2] = 0.248 mM, % loading = 23%, pH = 7.4, temp. = 25°C

3.9 Effect of cross-linker on drug release

In cross-linked polymeric structure the swelling process may be controlled by the introduction of an appropriate amount of a second monomer with hydrophobic character. Chemically cross-linked hydrogels as carrier for drugs were developed in the last decade [33]. Since cross-linker has pronounced effect on the swelling ratio as well as on kinetics of the drug release, in the present study EGDMA has been used as a cross-linking agent which is a known hydrophobic cross-linker. The effect of the degree of cross-linking on the swelling and drug release has been investigated in the range of 0.53–2.12 mM EGDMA in the feed mixture of the polymerization recipe. The results are shown in Fig. 7(a, b), which clearly indicate that, initially the swelling ratio and drug release increases up to 1.06 mM of EGDMA content, while beyond this concentration, both swelling ratio and drug release decrease. The observed increase is quite unusual and however, may be explained by the fact that due to hydrophobic nature of EGDMA the hydrophobic-hydrophobic interactions operative along EGDMA segments may cause loosening of the macromolecular chains of the nanoparticles which consequences results in larger release of the entrapped drug. The

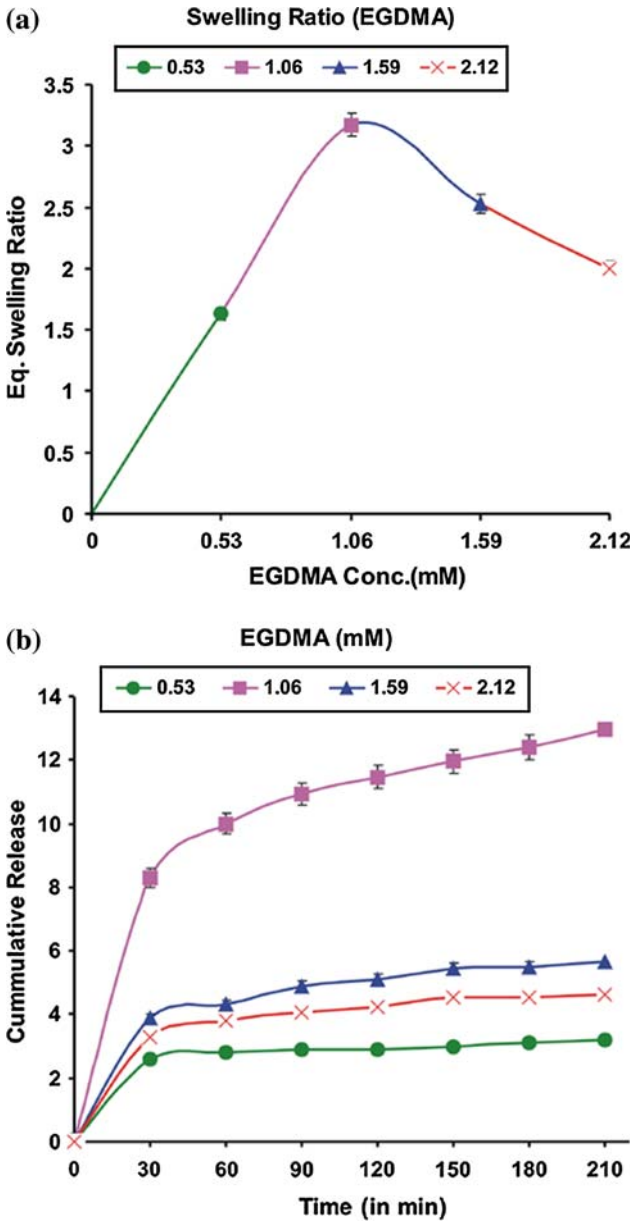

Fig. 7 Effect of cross-linker [EGDMA] content of the nanospheres on the **a** swelling profile and **b** release profile of 5-FU from loaded nanospheres of definite composition, [HEMA] = 12.37 mM, [Bz_2O_2] = 0.248 mM, % loading = 23%, pH = 7.4, temp. = 25°C

observed fall in the release beyond 1.06 mM of EGDMA could be attributed to the reason that high amount of cross-linker produces a compact network that increases the number of cross-links which consequently reduces the free volume accessible to the penetrant water molecule. Similar type of results has also been reported by other workers [34]. Some authors [35] have, however, reported that the introduction of cross-linker increases the glass transition temperature (T_g) of the polymer, which restrains the mobility of network chains at experimental temperature and, therefore, lowers both the amount of water sorption as well as drug release.

3.10 Effect of initiator on drug release

In free radical polymerization the concentration of initiator has a direct impact on the molecular weight of the polymer [36]. In the present case, Bz_2O_2 was used as a polymerization initiator and its concentration has been varied in the range of 0.082–0.330 mM in the mixture. The results are depicted in Fig. 8(a, b), which reveal that an initial increase in concentration of Bz_2O_2 in the range of 0.082–0.248 mM results in an increased swelling as well as drug release. A possible explanation may be that as the concentration of initiator increases the number of primary free radicals also increases which eventually results in lower molecular weight of the PHEMA. Since a polymer with lower molecular weight has lower hydrodynamic volume in aqueous solution, the PHEMA chains acquire greater mobility, and therefore, show increased swelling and drug release. However, beyond (0.248 mM) of initiator shorter PHEMA chains results in much smaller mesh size of the nanoparticles network, which might accommodate fewer amount of drug molecules and consequently may tend to lower amount of drug release.

3.11 Effect of pH on drug release

pH Responsive hydrogels constitute an important class of biomaterials which play a significant role in designing targeted drug delivery systems [33]. Several methods have been proposed for targeting the specific regions. Two of these, i.e. utilization of pH changes within the GI tract and exploitation of bacterial enzymes localized within the colon are of current interest in controlled drug delivery [36]. The wide range of pH allows a specific drug to be delivered to a target site only. The underlying principle for targeted drug delivery is the pH controlled swelling of hydrogel which normally results from the change in relaxation rate of network chains with changing pH of the medium. Some authors [37] have reported that under acidic condition, hydrogen bonded complexes may be formed between the monomeric units of the hydrogel.

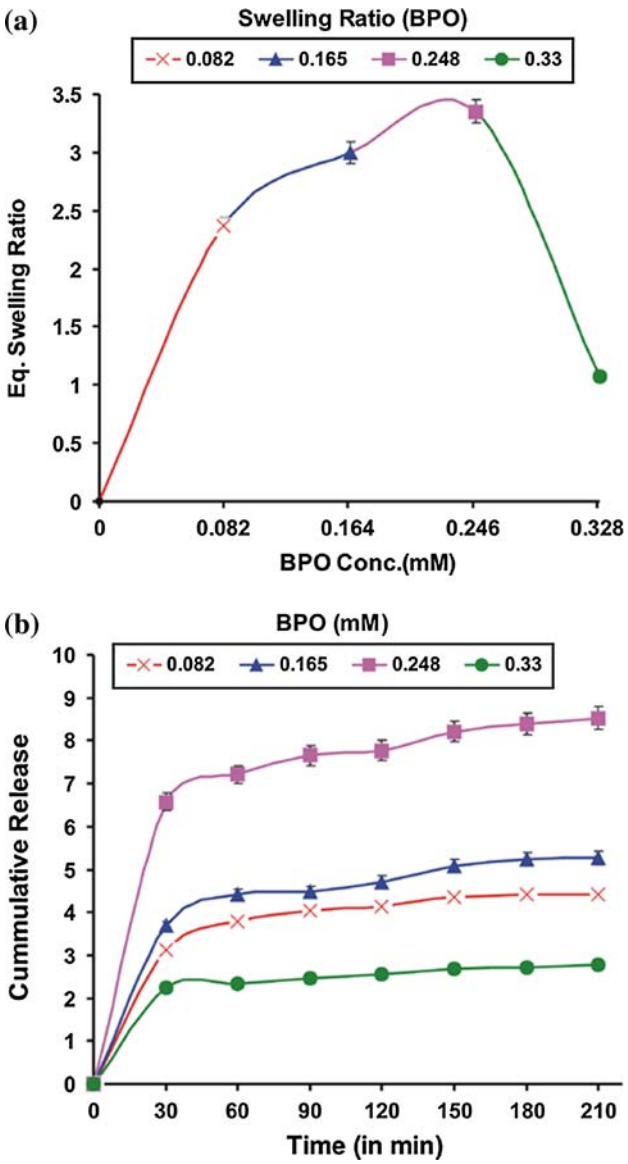

Fig. 8 Effect of initiator [Bz_2O_2] content of the nanospheres on the **a** swelling profile and **b** release profile of 5-FU from loaded nanospheres of definite composition, [HEMA] = 12.37 mM, [EGDMA] = 1.06 mM, % loading = 23%, pH = 7.4, temp. = 25°C

pH Sensitive polymers are used as drug releasing carriers via loading in aqueous solution. In the present work, the release dynamics of the 5-FU has been observed under varying pH conditions as found in GIT (e.g. stomach (gastric juice, 1.2), and small intestine (8.6)). The results depicted in Fig. 9(a, b), indicate a lesser drug release in acidic and alkaline solution while shows an optimum release in neutral (pH = 7.4) solution. The observed release results are consistent to the swelling results. In the acidic and alkaline pH, the nanoparticles do not swell sufficiently and as a result the drug loading decreased. Hence, under these conditions mostly the surface bound

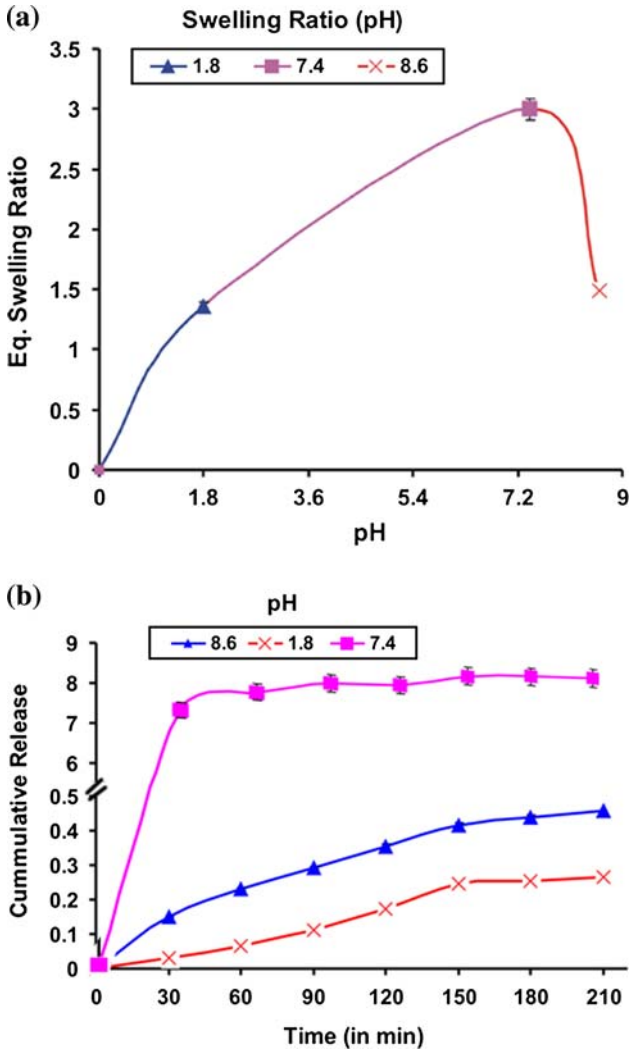

Fig. 9 Effect of pH on the **a** swelling profile and **b** release medium on the release profile of 5-FU from loaded nanospheres of definite composition [HEMA] = 12.37 mM, [EGDMA] = 1.06 mM, [Bz_2O_2] = 0.248 mM, % loading = 23%, temp. = 25°C

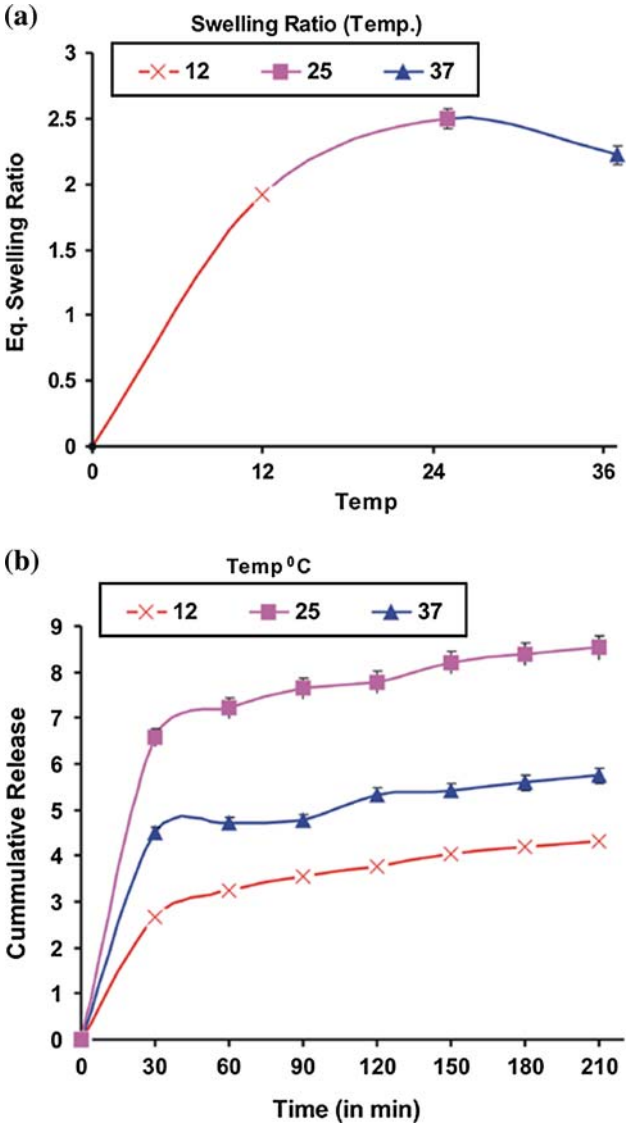

Fig. 10 Effect of temp. on the **a** swelling profile and **b** release medium on the release profile of 5-FU from loaded nanospheres of definite composition [HEMA] = 12.37 mM, [EGDMA] = 1.06 mM, [Bz_2O_2] = 0.248 mM, % loading = 23%, pH = 7.4

drug is released. However, at 7.4, pH, the nanoparticles swell and accordingly larger drug release was observed.

3.12 Effect of temperature on drug release

Temperature has a direct influence on the swelling behavior of a hydrogel as it affects both the segmental mobility of the hydrogel chains as well as the diffusion of penetrant molecules. In the present study, the effect of temperature on the swelling ratio and drug release through PHEMA nanoparticles has been investigated by varying the temperature of the swelling medium in the range of 12–37°C. The results are depicted in Fig. 10(a, b), which show that with increasing temperature, drug release increases up to 25°C, while above it a fall is observed.

The observed increase in the released amount of 5-FU up to 25°C can be explained by the fact that with increasing temperature, the network chains also undergo faster relaxation due to increased kinetic energy and thus facilitates the water sorption process [38]. The observed decrease, however, in release beyond 25°C may be attributed to the fact that with increasing temperature the H-bonds between the water molecules and network chains are broken, thus converting bound water into free water. This obviously results in a lower degree of swelling of nanoparticles and, consequently, a decrease in the released amount of 5-FU is observed.

3.13 Effect of physiological fluids

The influence of solute on the swelling behavior and release kinetics of 5-FU was examined by performing swelling and release experiments in the presence of solutes such as urea (15% W/V) and D-glucose (5% W/V) and in physiological fluids such as saline water (0.9% NaCl) and synthetic urine. The results are depicted in Fig. 11(a, b), which show that the presence of solutes suppresses the swelling ratio as well as drug release. The possible reason for the lower release of 5-FU in these solutes may be due to the presence of salt ions in the release medium which lowers the osmotic pressure in the system thus resulting in lower extent of swelling of loaded nanoparticles. Thus, obviously the nanoparticles with suppressed swelling will result in less amount of released 5-FU.

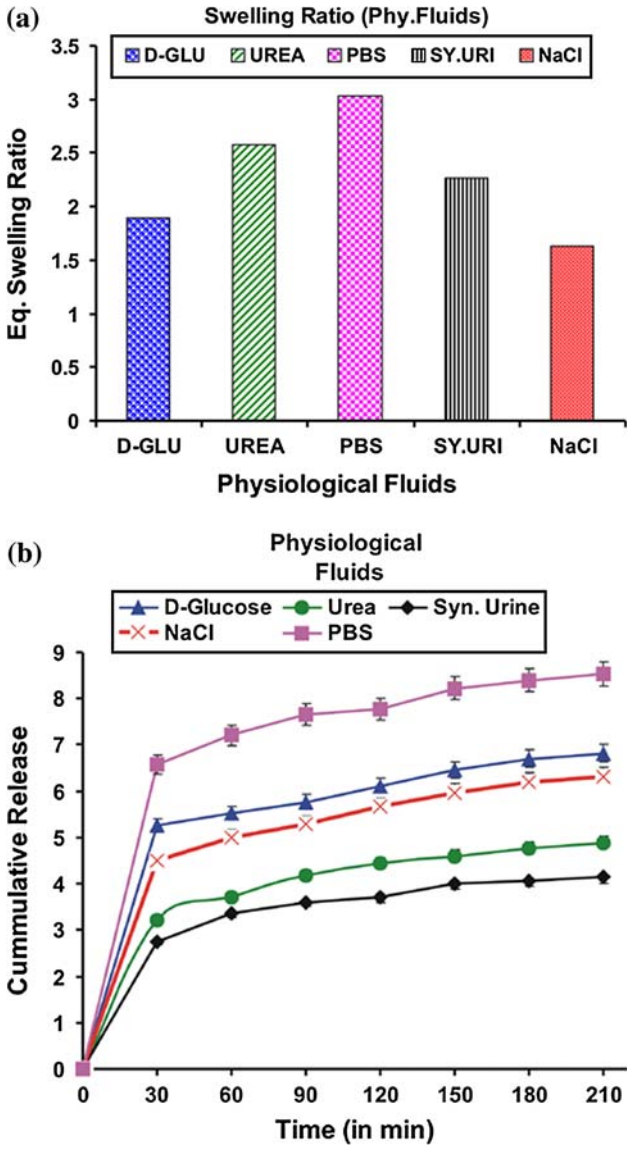

Fig. 11 Effect of phy. fluids on the **a** swelling profile and **b** release profile of 5-FU from loaded nanospheres of definite composition [HEMA] = 12.37 mM, [EGDMA] = 1.06 mM, $[Bz_2O_2]$ = 0.248 mM, % loading = 23%, pH = 7.4, temp. = 25°C

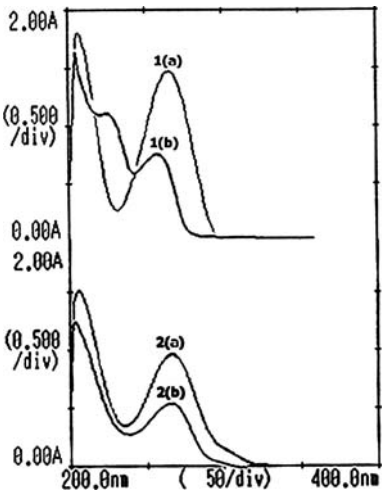

Fig. 12 UV spectra showing the chemical stability of 5-FU in its pure solution **a** and released media **b** at different pH (1.8(1), 7.4(2))

3.14 Chemical stability of drug

The chemical stability of the entrapped drug was investigated by recording the UV spectra of pure 5-FU in solution and released 5-FU at different pH of release media i.e. at pH 1.8 and 7.4. On comparing the spectra (Fig. 12) of pure and released drug solutions in different pH solutions it is revealed that the spectra are almost identical, and thus suggest that no significant change in chemical and bioactivity of the drug occurred following the loading and release of drug (5-FU).

4 Conclusions

Suspension polymerization of HEMA results in the formation of swellable nanoparticles which show promise to function as swelling controlled release system. The structural characterization of prepared nanoparticles by FTIR spectral analysis confirms the presence of functional groups of HEMA in the nanoparticles. The morphology of the nanoparticles as studied by SEM analysis suggests for an inhomogeneous surface and varying nanoparticles dimensions in the range 100–300 nm. The addition of model drug 5-FU, to polymeric nanoparticles proved to be successful with a 6–23% drug entrapment. It is found that release profiles of 5-FU are greatly influenced by varying the experimental parameters such as percent loading of 5-FU and concentration of HEMA, cross-linker and initiator.

With an increase in percentage loading of drug on to the nanoparticles, the swelling and release of 5-FU constantly increases. The released amount of drug constantly decreases with increasing HEMA content in the nanoparticles while in the case of cross-linker EGDMA the release of drug increases when the concentration of EGDMA is increased from 0.53 to 1.06 mM and decreases beyond 1.06 mM. Similarly when the concentration of initiator, benzoyl peroxide, is varied within the range of 0.082 mM to 0.330 mM, the drug release increases from 0.082 to 0.248 mM, while beyond 0.248 mM less drug release is noticed.

An optimum drug release is obtained near physiological pH (7.4) while lower release is noticed in acidic and alkaline pH range. A rise in temperature up to 25°C brings about an increase in the released 5-FU while a decrease is noticed beyond 25°C. The physiological fluids suppress the extent of drug release. The loaded drug shows retention of its chemical and bioactivity when released into PBS as release medium.

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