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Synthesis and *In Vitro* Release of Guest Drugs-Loaded Copolymer Nanospheres MMA/HEMA via Differential Microemulsion Polymerization

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ABSTRACT: The synthesis and characterization of different series of methyl methacrylate/hydroxyethyl methacrylate (MMA/HEMA) copolymeric nanoparticles with different monomer feed compositions and utilization of them in entrapment and controlling the release of hydrophilic drug (sodium warfarin) and hydrophobic drugs (ibuprofen and praziquantel) were investigated. The polymeric nanoparticles and their entrapment with drugs were prepared using oil-in-water (O/W) differential microemulsion polymerization technique in the presence of polyvinyl pyrrolidone and polyethylene glycol as biocompatible emulsifiers as well as ammonium persulfate as an initiator. The effect of HEMA content in the monomer feed composition on the colloidal properties was studied, and it is found that the particle size Dv, turbidity, and the negative charge increase with increasing of HEMA content but the surface tension decrease. Moreover, the entrapment efficiency (EE) is affected by the content of HEMA in the monomer feed composition, the drug hydrophobicity, and the monomer to drug ratio. It is concluded that, EE into MMA/HEMA in monomer feed composition as 90/10, 70/30, and 50/50 is found to be (95.3–98)%, (84–96.9)%, and (69.5–94.6)% for sodium warfarin (with high hydrophilicity) as well as, ibuprofen and praziquantel (with high hydrophobicity), respectively. The entrapment of drugs in polymeric nanoparticles is confirmed by IR-spectroscopy and transmission electronic microscopy. *In vitro* drug release experiments show that controlled release of drugs from copolymeric nanoparticles depend on HEMA content, the monomer to drug ratio, and the physiological pH.
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INTRODUCTION

During the last decade, polymeric nanoparticles have been developed as a viable and promising strategy for the biopharmaceutical industry. They fulfill the requirements of the drug carrier system to be an efficient and versatile drug carrier that are: (i) particle size in the submicron range, (ii) the possibility of surface modifications, (iii) high drug-loading capacity, (iv) colloidal stability of the latex in biological media, and (v) the lack of toxic side effects induced by the carrier or additives.[1]

Drug loading into the polymeric nanoparticles PNPs is achieved by two methods: by incorporating the drug at the time of NP production or, by adsorbing the drug after the formation of NPs by incubating them in the drug solution. It is evident that a large amount of drug can be entrapped by the incorporation method when compared with the adsorption.[2,3] Drugs can be incorporated in polymeric nanoparticles either in physical or chemical methods. However, the physical methods involve high cost and complicated processes. In addition, only few polymers are suitable for such treatment.[4]

One of the successful chemical methods of incorporation of drugs in nanoparticles can be achieved through microemulsion polymerization of a suitable monomer–emulsifier system. Microemulsion systems appear the most promising technologies as drug delivery systems, where these systems show high drug entrapment and release under sink conditions, and they satisfy most of the required criteria for fruitful uses in pharmacy that are tolerance toward additives, small size, biodegradability, and easy elimination from the body[5,6]; as well as spontaneous formation, ease of manufacture with little energy input, stable over a wide temperature range, improved solubilization of bioactive materials, and optical transparency with low viscosity.[7] In addition, the nanosized droplets with an enormous increasing of the interfacial area are an important factor in sustained and targeted drug delivery.[8,9]

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However, two main drawbacks limit its broad applications: (1) a high emulsifier-to-monomer ratio, usually larger than 1, and (2) a low monomer concentration with respect to water (low polymer content of the final latexes) is usually less than 10 wt %.[10–13] Consequently, many efforts have been undertaken to obtain nanosized latexes containing higher polymer contents at lower emulsifier concentration. One approach is to increase the amount of polymer produced for a given amount of emulsifier with semicontinuous (modified) or continuous (differential) microemulsion polymerization technique.[14] Modified microemulsion polymerization involves adding monomer directly dropwise to a prepolymerized microemulsion,[15–18] whereas differential microemulsion polymerization technique involves adding monomer continuously to a preheated mixture containing a designed amount of initiator, emulsifier, and water.[19–21]

A number of polymers, both synthetic and natural, have been utilized as drug delivery devices. Among synthetic polymers, poly(methyl methacrylate) that was the first successful acrylic polymer used as a biomaterial,[22] but (PMMA) particles have been reported to show slow biodegradability. The biodegradability can be improved through the copolymerization of MMA with 2-hydroxyethyl methacrylate (HEMA), which forms a more biodegradable polymer.[23,24] Poly(MMA/HEMA) copolymers have stimulated increasing interest and research attention in recent years because of their biocompatibility and water insolubility. Besides, the main advantage of poly(MMA/HEMA) copolymers is that both the hydrophilic and the hydrophobic drugs can be incorporated, also have excellent chemical stability because of their three-dimensional polymeric networks.[25] All these favorable properties described above make poly(MMA/HEMA) microspheres extremely valuable for various applications in therapeutical and biotechnological fields, such as cell immobilization,[26] drug delivery systems,[27,28] and packing materials in chromatography.[29]

Moreover, using biocombatible emulsifier, as polyvinyl pyrrolidone (PVP) and polyethylene glycol (PEG), enables pharmaceutical application without needing for excess purification, where PVP is approved by the U.S. Food and Drug Administration FDA as a biocompatible and nonantigenic compound.[30–32]

Bhawal et al.[23] reported previously entrapment of a hydrophobic drug during O/W emulsion polymerization of MMA and HEMA stabilized by SDS resulting in entrapment efficiency (EE) range as (61.2–83%) depending on HEMA content in the monomer feed composition but through unstable latex with large particle size in the presence of coagulation.

However, the objective of this work is to synthesis of MMA/HEMA with three different monomer feed compositions as 90/10, 70/30, and 50/50, and their entrapment with both hydrophilic (sodium warfarin) and hydrophobic (ibuprofen and praziquantel) drugs to copolymeric nanospheres through differential microemulsion copolymerization technique in the presence of PVP/PEG as biocompatible emulsifier, resulting homogeneous stable latex with nanosized particle without any coagulation. This work is extent to investigate the effect of HEMA content in the monomer feed composition on the colloidal properties via measurement of the average particle size Dv, turbidity, surface tension, and zeta potential. As well as, studying the effect of drug hydrophobicity, HEMA content in the monomer feed composition and drug content on the drug loading and the *in vitro* drug release through simulated intestinal fluid (pH 7.4) and in simulated gastric fluid (pH 1.2), at 37 ± 0.5°C.

EXPERIMENTAL

Materials

The monomers, methyl methacrylate (MMA; Across company, New Jersey, USA) and 2-hydroxyethyl methacrylate (HEMA; Sigma company, Germany), were excluded of inhibitor via filtration through an active alumina column[32] and silica gel column, respectively. Purified monomers were stored in a dark container, refrigerated and used within 1 month of purification. Polyvinyl pyrrolidone (PVP, Mwt. = 40,000; Bioshop, Canada) was used as received without further purification. Ammonium persulfate (APS; BDH laboratory Supplies Poole, BH15 1TD, England) was used as water soluble initiator. Polyethylene glycol (PEG: M.wt. = 300) was purchased from Hoba chemie -Bombei-India. Ethylene glycol dimethacrylate (EGDMA) as cross-linking agent was purchased from Merck, Germany. Drugs, Sodium warfarin, Ibuprofen, and Praziquantel were provided as gift from GlaxoSmithKline Company, Egypt. Ethyl alcohol (absolute) and hydrochloric acid are pure reagent for analysis and supplied by El-Nasr Pharmaceutical Chemical Company, Egypt. Dodeca-Phosphotungstic acid A. R. was purchased from Nen Tech Ltd. Brixworth Northants. U.K. Potassium dihydrogen phosphate (KH_2PO_4) was purchased from GenLab (packaged in Egypt), and sodium hydroxide (NaOH) (pellets) 99% and potassium chloride were from Modern Lab, Egypt, and both are used as received. Double distilled water was used in all experiments.

Methods

Differential Microemulsion Polymerization. Poly(MMA/HEMA) were synthesized by a continuous process in a ternary oil-in-water (O/W) microemulsion system containing 10% monomer concentration stabilized by PVP (1.5×10^{-3} g.mol/L) in conjunction of PEG (74×10^{-3} g.mol/L) as biocompatible emulsifiers. The copolymers were prepared with monomer feed composition MMA/HEMA as 90/10, 70/30, and 50/50. Ammonium persulfate APS was used as an initiator in concentration of 0.0228 g.mol/L. The stirring was kept at ~ 350 rpm during the whole process.

In a typical procedure, the experiment was carried out as follows[17]: in a 250-mL three-necked round-bottomed flask, equipped with reflux condenser, the emulsifier dissolved in 30 mL distilled water was overnight mechanically stirred at room temperature. Then, the weighed initiator was dissolved in 15 mL water and the first portion of this solution (30%) was added to the reactor and left in water bath at 65°C in the presence of pure nitrogen gas. When the temperature reaches the decomposition temperature of the initiator (65°C), the desired monomers concentration as well as the second portion of initiator (60%) was added to the aqueous phase dropwisely through a period of 1 h. The third portion of the initiator (ca 10%) was added, and the reaction content was left for another 2 h to complete the polymerization.

Entrapment of Drugs Through Differential Microemulsion Polymerization. Each of different monomer feed composition of (MMA/HEMA) as 90/10, 70/30, and 50/50 is entrapped by each of

(sodium warfarin) and hydrophobic drugs (ibuprofen and praziquantel) in monomer to drug ratios as 20 : 1, 10 : 1, and 6 : 1.

Entrapment of hydrophilic drug. An appropriate amount of the drug as water soluble drug[34] was dissolved in the aqueous solution of the emulsifier (water phase) with the first portion of the initiator before polymerization, and then the differential microemulsion polymerization was preceded as described previously by adding each of the monomer and the remaining initiator dropwisely at the reaction temperature through dropping funnel.

Entrapment of hydrophobic drug. The hydrophobic drug (Ibuprofen or praziquantel) was dissolved in the monomer phase before polymerization through the differential microemulsion polymerization. The crosslinking agent ethylene glycol dimethacrylate has also been included along with monomer for polymerization in a ratio 2% of the total monomers.[23] When the temperature reached the decomposition temperature of the initiator (65°C), the monomer including the drug and the crosslinker were dropped slowly to the emulsifier solution (aqueous phase) through dropping funnel as mentioned obviously in the differential microemulsion technique.

Solid Content and Conversion Measurement. Both of the solid content of the nanolatexes and the polymerization conversions for all samples are determined gravimetrically by a weighing method. The polymerization conversion is calculated by

$$\text{Conc.}\% = \frac{[P]}{[M]} \times 100$$

where, $[P]$ is the weight of the dry polymer and $[M]$ is the weight of the introduced monomer mixture.

However, the solid content (S%) of the polymer latex calculated by:

$$S\% = W_1/W_2 \times 100\%$$

where W_1 and W_2 are the weights of the dry polymer and the polymer latex, respectively.

Surface Tension Measurement. The liquid-vapor surface tensions of the resulting nanolatexes were measured at room temperature using a K9 tensiometer (Krüss, Germany) (Optisch-Mechanische werkstätten Humberg39, Germany) based on the Lecomte de Noüuy method using a rigid *O*-platinum ring.[35] For each emulsifier type, the measurements were done on the prepared polymer latex. Careful cleaning was done after each change of polymer latex. After the *O*-ring had been immersed into a sample solution and allowed to stay, there the reading was taken and recorded. The mean value of three measurements for each sample was taken and registered.

Particle Size and Morphology Analysis. The particle size of the nanoparticles was measured by transmission electronic microscopy (TEM), where the TEM images were obtained by (JEM-1230-electron microscopy operated at 60 KV). Before taking a TEM image the sample was diluted at least 10 times by water. A drop of well dispersed diluted sample was placed onto a copper grid (200 mesh and covered with a carbon membrane) and dried at ambient temperature. A drop of phosphotungestic acid (0.4%) as a stain was deposited over the dried sample.[36] The particle size was taken from an average of 15-particles.

Turbidity Measurement. The turbidity of the prepared latexes was measured as a function of the effect of the change in the 2-hydroxyethyl methacrylate ratio in the monomer feed composition. It was evaluated with a HANNA instrument model HI98703 portable turbidimeter.[37] The polymer latex samples were diluted to 5% using distilled water and measured in a cylindrical glass cell. The turbidity[38] of the emulsions was characterized by a turbidity index, $T = -\log(I_0 - I)$, where I_0 is the intensity of the incident light and I is the intensity of the transmitted light. The optical absorbance was measured immediately and recorded.

Zeta Potential Measurement. The electrophoretic mobility (μ_e) of the latex particles was measured at various HEMA ratios using the ZetaSizer from Malvern Instruments (3000-HS model). The zeta potential (ζ)[39] was calculated from the electrophoretic mobility using the Smoluchowski's equation,

$$\zeta = \eta/\varepsilon * \mu_e$$

where η is the viscosity and ε the permittivity of the medium For zeta potential measurement, sodium chloride solution of 10^{-3} mol/L was used.

Drug EE. To determine the drug EE, the content of drug in polymers was determined by an indirect method,[40] through measuring the free drug (unloaded drug). The unloaded drug was collected from the nanoparticles stable dispersion by dissolving in ethanol then free drug was determined in the clear supernatant following separation of nanoparticles by a combined ultracentrifugation technique at 50,000 r.p.m. for 30 min, then the drug concentration in the solution was determined by measuring the absorbance at 250, 270, and 210 nm for sodium warfarin, ibuprofen and praziquantel, respectively, on a Shimadzu Ultraviolet–visible spectrophotometer using a standard calibration curve experimentally obtained with ethanol solutions. The drug EE was defined as the ratio of the weight of the drug entrapped in the polymeric nanoparticles to the weight of the drug initially used.[41]

FTIR Spectroscopy. FTIR spectrum of the copolymer free from drug, loaded with drug and free drugs was recorded on a FTIR spectrophotometer (Thermo Nicolet, NEXUS, TM) in the range of 4000–400 cm^{-1} using KBr pellets.

***In Vitro* Drug Release Studies.** Buffer solution of pH 1.2 (simulated gastric fluid) was prepared by mixing 250 mL of 0.2*M* HCl and 147 mL of 0.2*M* KCl. Buffer solution of pH 7.4 (simulated intestinal fluid) was prepared by mixing 250 mL of 0.1*M* KH_2PO_4 and 195.5 mL of 0.1*M* NaOH. *In vitro* release studies were carried out in simulated intestinal fluid phosphate buffered saline media of pH 7.4 and simulated gastric fluid at pH 1.2 using the dialysis bag technique.[42,43] Dialysis sacs were equilibrated with the dissolution medium for few hours before experiments. A total of 0.5 g of polymer–drug in 5 mL of buffer solution was taken in the dialysis bag. Dialysis bag was dipped into receptor compartment containing 100 mL dissolution medium, which was (mild stirred magnetically) at 37 ± 0.5°C.

Table I. Characterization of the Polymeric Nanoparticles Synthesized Through (O/W) Differential Microemulsion in the Presence of PVP/PEG as Biocompatible Emulsifier. ($n = 15$)

Monomer composition MMA/HEMA	Solid content (%)	Surface tension (mN/m)	Particle size (nm)	Turbidity (NTU)	Zeta potential (mV)
90/10	12	49.5	40 (±8)	317	−1.03
70/30	16	44.5	53 (±7)	467	−4.624
50/50	16.8	37.6	92 (±8)	610	−6.366

The receptor compartment was closed to prevent the evaporation losses from the dissolution medium. A total of 5 mL of sample was withdrawn at regular time intervals, and the same volume was replaced with a fresh dissolution medium. Samples were analyzed for drug content by UV–vis spectrophotometer. These studies were performed in triplicate for each sample, and the average values were used in data analysis.

Statistical Analysis. All the tests including determination of drug content and *in vitro* drug release were carried out in triplicate and the averages were reported. Statistical data analysis was performed using the Student's *t*-test with $P < 0.05$ as the minimal level of significance.[44] Error bars on graphs represent standard errors. The average particle sizes were the average size of 15 particles.

RESULTS AND DISCUSSIONS

The polymeric nanoparticles of (MMA/HEMA) in ratios as 90/10, 70/30, and 50/50 were synthesized through (O/W) differential microemulsion polymerization technique using of PVP in conjunction with PEG as biocompatible emulsifiers as well as ammonium persulfate as initiator at 65°C. Generally, prior polymerization, the samples were transparent at the reaction temperature, and as the polymerization proceeded, the mixtures developed a bluish tint or became translucent indicating the presence of slightly larger colloidal particles. Differential microemulsion technique permitted in increasing HEMA content in the monomer feed composition upto 50% producing nanoparticles almost uniform in size, and there are no aggregates. However, Özer et al.[45] found that increasing HEMA percentage over HEMA/MMA10/90 through batch microemulsion polymerization caused a significant change in the size distribution and formation of very large particles and even agglomeration in the medium took place. Also, Bhawal et al.[23] noted the presence of coagulation in the polymerization of HEMA/MMA in a ratio of 30/70 through emulsion polymerization and also causing very large particle size.

Therefore, the content of HEMA in the monomer feed composition plays an important role on the colloidal properties, and this is examined by measurement of turbidity, surface tension, and zeta potential as well as the average particle size and the extent of this role is to affect on the drug EE and *in vitro* drug release.

Influence of HEMA Content on the Colloidal Properties

Table I gives the colloidal properties through measurements of solid content, the surface tension of the latex, the average particle size, the colloidal aspects or the turbidity of the latex, and the zeta potential at different HEMA contents in the monomer feed composition (MMA/HEMA as 90/10, 70/30, and 50/50).

Initially, both of the solid content of the nanolatexes and the polymerization conversions for all samples are determined gravimetrically, by a weighing method. It is found that polymerization using PVP/PEG produced latex with conversion up to 98%. However, the solid content values are registered in Table I and it is noted that the solid content increases with HEMA content and it can be explained through increasing of HEMA over 10% leads to network formation.

The effect of HEMA content in the monomer feed composition on the colloidal properties of the latex was studied in terms of the liquid-vapor surface tension of the latex, the average particle size, the colloidal aspects or the turbidity of the latex, and the zeta potential of the latex particles. The data are registered in Table I. These data show that the surface tension decreased with increase of HEMA content in the monomer feed composition which may be indicated to progress of the air–water interface saturation by polymer molecules with increase of HEMA content.[46]

On the other hand, it is noticeable that the average particle size increases with increasing HEMA content which may be attributed to the difference in the monomer partitioning of MMA and HEMA in the different phases involved in the particle formation and the subsequent stabilization of the particles by the available emulsifier. MMA is less soluble in water than HEMA; therefore, its partitions are mainly in the micelles. HEMA partitions between the aqueous phase and the micelle–water interface, and the water soluble initiator generates free radicals in the aqueous phase and the polymer chains grow up to a critical length until they become surface-active. At this point, the probability of these surface-active free radicals entering the micelles is very high because of their higher residence time at the micelle–water interface that results in micellar nucleation. Therefore, any excess in HEMA content leads to further increase in the average particle size. The further addition of monomer units beyond the critical chain length results in their precipitation in the aqueous phase; this is better known as homogeneous nucleation.[23,45]

In addition, the turbidity of the obtained microemulsion was measured as a function of the effect of the change in HEMA content. As expected, the turbidity increased with increasing

HEMA content and elucidates the increase in the particle size as HEMA content increased. Increasing in HEMA concentration leads to a shift in particle formation, mainly through a coagulative nucleation mechanism,[23] resulting in greater particle size and higher turbidity. Also, the polymer formed during the polymerization decreases the ordering in the microemulsion system and this is reflected in an increased scattering intensity (or turbidity). This behavior can simply be explained by the increased attraction between droplets (particles).[47]

Moreover, zeta potential (ζ) measurements are required to show the effect of HEMA content on the particle charges. Generally, the data presented in Table I showed that the prepared microemulsion lattices using PVP/PEG as emulsifier have low negative zeta potential indicating to the stability of the obtained latex. However, it is noted that further increasing of the HEMA content in the monomer feed composition concentration led to an increase in the (ζ) values. The higher zeta potential obtained only reflects the increase of the surface charge upon HEMA incorporation with the presence of an extra hydroxyl group in every repeating unit.[39]

Drug EE

Both of the hydrophilic sodium warfarin drug (an anticoagulant drug) as well as the hydrophobic ibuprofen (non-steroidal anti-inflammatory drug) and praziquantel drugs (antiparasite drug) were entrapped through differential microemulsion copolymerization of (MMA/HEMA) in the presence of biocompatible emulsifiers (PVP/PEG) producing copolymeric nanospheres with monomer feed composition of MMA/HEMA as 90/10, 70/30, and 50/50, with three different drug content in monomer to drug ratios as 20 : 1, 10 : 1, and 6 : 1.[48]

The entrapped amount of each drug was quantified by an indirect method as depicted in the experimental part. The loading efficiency or EE depends on drug type, monomer composition, and polymer to drug ratio. It can be calculated as follows:[49,50]

$$\text{Entrapment Efficiency} = \frac{\text{actual weight of the drug in sample}}{\text{theoretical weight of the drug}} \times 100$$

Table II provides the EE values and their relation with both of the monomer feed composition and the monomer to drug ratio for the various drugs used. Generally, these data showed that the technique of differential microemulsion polymerization of MMA and HEMA is an excellent method to incorporate both hydrophilic and lipophilic drugs producing drug-loaded polymeric nanospheres with high drug entrapment amount.

However, the drug EE is very sensitive to the drug type and this fact was confirmed previously by Zhang et al.[51] It was known that absorption of drugs from a microemulsion formulation is influenced by several factors such as particle size and the partition coefficient of the drug between the two immiscible phases.[5] Therefore, the small particle size of the copolymeric nanoparticles (presented in Table I) produced from differential microemulsion polymerization technique may also made the copolymer more susceptible to drug percolation, producing high EE values.[45]

Table II. The Relationship Between the Entrapment Efficiency EE and Both of Monomer Feed Composition and Drug Content (Monomer to Drug Ratio) for Each of Sodium Warfarin, Ibuprofen, and Praziquantel at Constant Concentration of Emulsifier (PVP/PEG: 1.5/74 × 10^{-3} g.mol/L) and Initiator (APS: 0.0228 g.mol/L). (±S.D., n = 3)

Monomer composition	Entrapment Efficiency (EE)									
	Sodium warfarin Monomer:drug			Ibuprofen Monomer:drug			Praziquantel Monomer:drug			
MMA/HEMA	20 : 1	10 : 1	6 : 1	20 : 1	10 : 1	6 : 1	20 : 1	10 : 1	6 : 1
90/10	95.3(±0.01)	96.7(±0.01)	98(±0.01)	84(±0.014)	91.5(±0.015)	96.7(±0.01)	69.5(±0.02)	85.9(±0.02)	94(±0.015)
70/30	95.5(±0.012)	96.7(±0.02)	98(±0.012)	85(±0.012)	91.7(±0.02)	96.9(±0.01)	71.5(±0.015)	86(±0.024)	94.4(±0.01)
50/50	93.5(±0.02)	97(±0.024)	98(±0.01)	86(±0.02)	91.8(±0.017)	96.9(±0.01)	75(±0.027)	86.3(±0.018)	94.6(±0.02)

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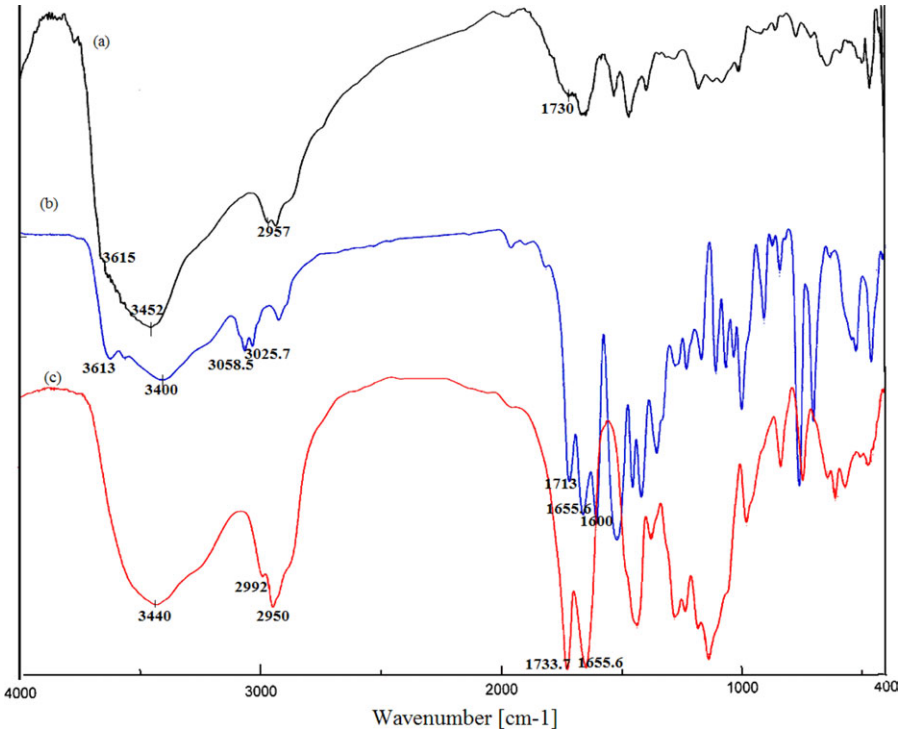

Figure 1. FTIR spectra of (a) copolymeric nanospheres of MMA/HEMA70/30 without drug, (b) free sodium warfarin, and (c) sodium warfarin-loaded polymeric nanospheres. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Since the partition- (P) or distribution coefficient (D) is the ratio of concentrations of a compound in the two phases of a mixture of two immiscible solvents at equilibrium,[52] the drug EE in the copolymer (MMA/HEMA) nanoparticles appeared to be governed by the partition coefficient of the drug,[53] where the hydrophylic sodium warfarin [with low partition coefficient value as ($\log P = 0.38-1.6$)[54,55]] exhibited higher loading in the polymeric nanoparticles than both of the lipophylic ibuprofen and praziquantel [with high partition coefficient value as ($\log P = 2.12-2.48$)[56,57] and ($\log P = 2.5$),[58] respectively]. It was noted that sodium warfarin exhibits the highest values of EE presented in Table II in range of 93.5–98%, whereas praziquantel showed the lowest values of EE ranging in 69.5–94.6%. Moreover, the entrapment of ibuprofen shows also high values in the range of 84–96.9% by comparing with the results of Thompson et al.,[50] where they incorporated ibuprofen drug in microspheres by emulsion solvent evaporation (ESE) method and reached to the best condition of EE of 68%, whereas Sivakumar and Rao[40] loaded ibuprofen in percentage of 48.7% through poly(MMA-HEMA) core–shell hydrogel microspheres.

In addition, drug EE values presented in Table II were found to be dependent on the amount of drug initially introduced where it increased upon increasing the drug concentration. This was investigated using monomer to drug ratios as 20 : 1, 10 : 1, and 6 : 1 for polymerization of the microemulsion templates, and the EE values tend to ~98, 96.9, and 94.6% for sodium warfarin, ibuprofen and praziquantel, respectively.

Drug EE may be influenced by the composition of the polymer, so, variation of HEMA content can affect the amount of loaded drug at low drug content (monomer: drug 20 : 1), the (EE) increased with HEMA content in both ibuprofen and praziquantel. These results were confirmed previously by other authors,[23,40] where they found that the EE increased with HEMA content. However, at high drug content, the composition of the copolymer (MMA/HEMA molar ratio) did not appear to affect the EE and depends only on the drug content.[53]

Therefore, the differential microemulsion polymerization technique is an excellent method for entrapment high amounts of both hydrophylic and lipophylic drugs in the copolymeric nanospheres MMA/HEMA. On the contrary, in a previous study[58,59] only moderate entrapment efficiencies were found where they studied the entrapment of insulin in the batch microemulsion system through interfacial polymerization and found that the EE was 52% in the best condition. However, they generally found an increase in entrapment of insulin with increasing drug content.

FTIR Spectroscopy

The FTIR spectra of the (MMA/HEMA) copolymeric nanospheres with monomer feed composition as MMA/HEMA70/30, free drugs and each of drug-loaded polymeric nanospheres with the corresponding monomer composition are shown in Figures 1–3 and indicated the details of functional groups present in each one.

Representative FTIR spectra (a) of free (MMA/HEMA) copolymers are presented in Figure 1. The (MMA/HEMA) copolymer spectrum contains strong adsorption at 2950 and 1730 cm^{-1} that corresponds to aliphatic C—H and carbonyl C=O stretches, respectively. Several medium to strong bands in the 1610–1300 cm^{-1} region were due to CH_3 and CH_2 deformations, and two

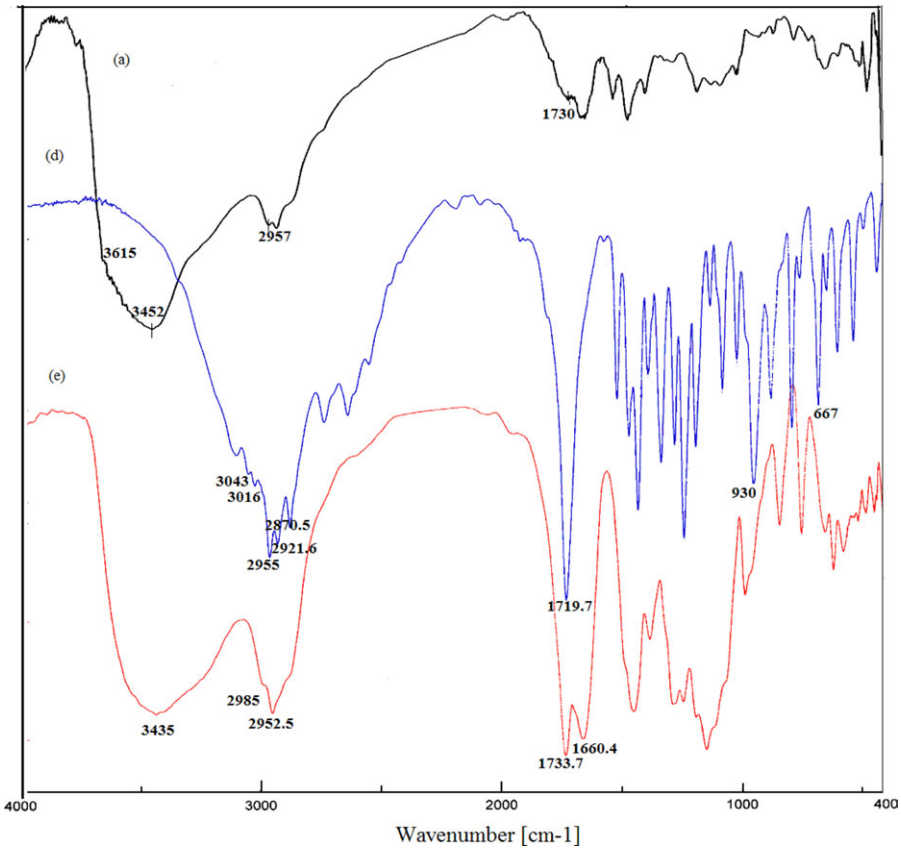

Figure 2. FTIR spectra of (a) polymeric nanosphere of MMA/HEMA70/30 without drug, (d) free ibuprofen, and (e) ibuprofen-loaded polymeric nanosphere. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

strong bands at 1068.4 and 998 cm^{-1} were due to C—O—C asymmetric and symmetric vibrations, respectively. In addition the presence of strong absorption at 3615 cm^{-1} and 3452 cm^{-1} that corresponded to O—H and C—H stretching vibrations, respectively. Bands appearing at 1068, 1261 cm^{-1} correspond to ester stretching vibration of acrylate polymer. However, the spectra (b) of free sodium warfarin is characterized by the presence of strong absorption at 3553, 3400.85 and 3613 cm^{-1} corresponded to O—Na and C—H stretching vibrations, respectively. The bands at 3058.5 and 3025.7 cm^{-1} refer to the aromatic C—H stretch in addition 1713.4, 1655.6 and 1600 cm^{-1} are attributed to the aromatic C=C bending.

By comparing the IR spectra of the sodium warfarin-loaded copolymer (c) with that for both free copolymer (a) and free sodium warfarin (b), it was noted that the presence of details of the main functional groups bands in both copolymer and sodium warfarin. IR spectra of the drug-loaded copolymer (c) in Figure 1 contain bands of —OH and —COO— at 3440 and 2950 cm^{-1} in more intense than that of the free one. Band at 2992 cm^{-1} refers to aromatic C—H stretch. In addition, bands corresponding to the aromatic C=C bending at 1733.7 and 1655.6 cm^{-1} that are not found in the IR spectra of the free copolymer (a) but found in the IR spectra of free sodium warfarin.

With respect to Ibuprofen, spectra (d) for ibuprofen as free drug in Figure 2. It is noted that the bands appearing at 3043, 930–667, 1719.7 and 1660 cm^{-1} are corresponding to aromatic C—H stretch, aromatic C—H bending, and aromatic C=C bending, respectively. In addition, bands at 3016, 2955, 2921.6 and 2870.5 cm^{-1} correspond to the carboxylic —COOH groups.

However, the IR spectra (e) of the ibuprofen-loaded copolymer (Figure 2) contain details of all bands that characterize both free polymer (a) and free ibuprofen (d). It shows clearly the function groups of the —OH band of HEMA in the copolymer at 3435 cm^{-1} and the —COOH band of ibuprofen at 2985 and 2952.5 cm^{-1}, beside the other bands that characterize both free agents (the copolymer and ibuprofen).

Figure 3 shows the IR spectra (f) of free praziquantel that contains the details of functional groups present in the structure of this drug. The presence of strong absorption at 2928 cm^{-1} and 2853 cm^{-1} corresponds to the two amide groups —CON— and aromatic C—H stretch. In addition, a sharp intense band was found at 1650 cm^{-1} corresponding to aromatic C=C bending.

However, the IR spectra (g) of the praziquantel-loaded copolymer (Figure 3) show all the bands that characterize both polymer and the drug. The —OH band of HEMA in the copolymer is at 3428 cm^{-1}, the function groups of the —CON— band of praziquantel at 2952.5 cm^{-1}, in addition to the bands at 1731.7 and 1660 cm^{-1} corresponding to aromatic C=C bending are clearly found.

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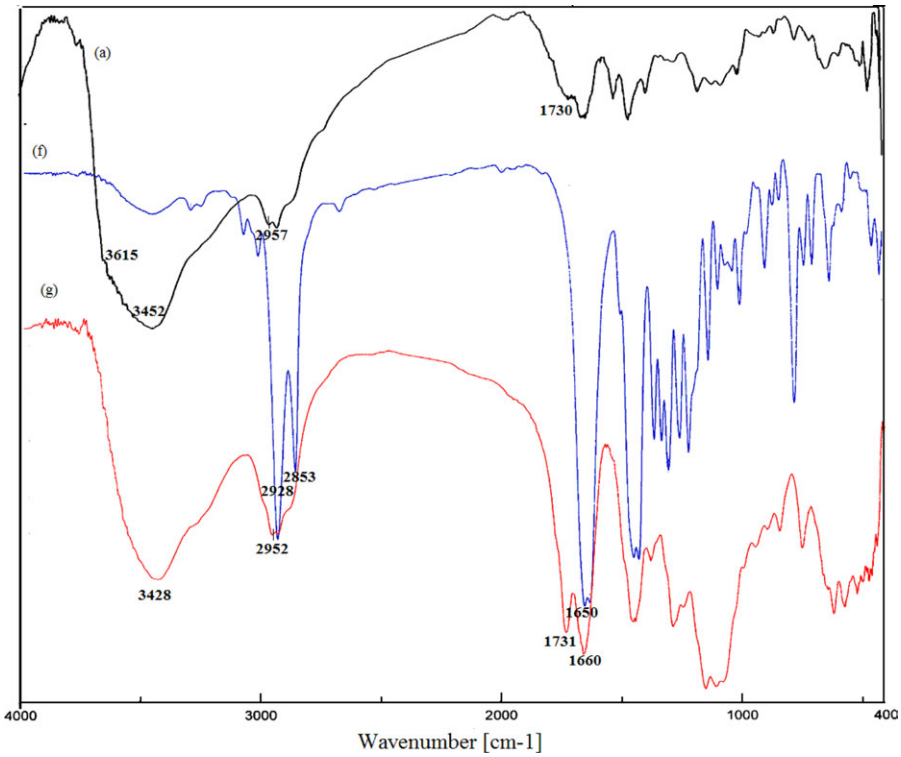

Figure 3. FTIR spectra of (a) polymeric nanosphere of MMA/HEMA70/30 without drug, (f) free praziquantel, and (g) praziquantel-loaded polymeric nanosphere. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The IR spectroscopic data clearly indicated the presence of both drug and poly(MMA/HEMA) copolymer in the nanosphere structure.

Morphology Analysis

Figure 4 showed images of transmission electronic microscope TEM of the obtained copolymeric nanoparticles with monomer feed composition of MMA/HEMA 50/50 without and with loaded drugs, including sodium warfarin, ibuprofen, and praziquantel. These images showed spherical particles with almost a similar size (narrowly size distributed) and showed both smooth and ridged surfaces for the polymer. By comparing morphologies of the polymer without and with loaded drug, TEM observation suggested that the entrapment of drugs can trigger a significant morphological transformation, where an occurrence of morphology transformation means a higher drug loading, and vice versa.[51] However, it was noted that the morphology transformation for polymer entrapped with praziquantel is less than that occurred in the other two cases referring to less loading amount that confirms the low EE values for praziquantel. In addition, the average particle size Dv of the produced drug-loaded polymeric nanospheres are presented in Table III and showed slightly increase with loading to 94 and 95 nm for both sodium warfarin and ibuprofen, respectively, but for praziquantel Dv increased to 126 nm producing drug-loaded nanospheres.

In Vitro Drug Release Studies

In vitro drug release was studied as a function of many factors as the effect of change of HEMA content, pH of the dissolution media, and drug content for the three applied drugs either hydrophilic or hydrophobic. The drug release studies involve

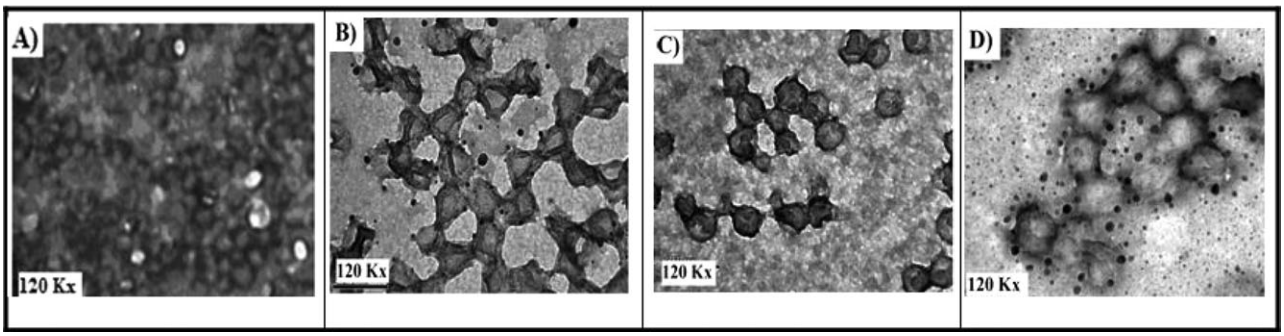

Figure 4. Typical TEM micrographs of polymeric nanoparticles with monomer feed composition as MMA/HEMA 50/50 without and with drugs at magnification of 120kx where a) copolymeric nanoparticles, (b) Sodium warfarin-loaded copolymeric nanoparticles, (c) Ibuprofen-loaded copolymer nanoparticles, and d) Praziquantel-loaded copolymeric nanoparticles.

Table III. The Average Particle Size of the MMA/HEMA Copolymeric Nanospheres Without and with drug Loaded for Each of Sodium Warfare, Ibuprofen, and Praziquantel. (n = 15)

Feed monomer composition MMA/HEMA	Particle size Dv (nm)			
	Copolymer	Sodium warfarin-entrapped copolymer	Ibuprofen-entrapped copolymer	Praziquantel-entrapped copolymer
50/50	92 (±8)	94 (±9)	95 (±7)	126 (±9)

copolymeric nanospheres with three different HEMA content in the monomer feed composition as MMA/HEMA90/10, 70/30, and 50/50 loaded with one of the three drugs studied sodium warfarin, ibuprofen, and praziquantel. These studies were carried out by suspending the sample (through dialysis bags) in two different physiological pH media, in simulated intestinal fluid (pH 7.4) and in simulated gastric fluid (pH 1.2), at 37 ± 0.5°C.

Figures 5–7 present the cumulative drug released % as a function of time from copolymeric nanoparticles made from the three different monomer feed composition for each of sodium warfarin, ibuprofen, and praziquantel, respectively. These figures show controlled release of the drugs from copolymeric nanoparticles; generally, smaller particle size nanoparticles show rapid initial release or burst that is mainly attributed to weakly bound or adsorbed drug to the large surface of nanoparticles.[61] The degree of burst release will generally depend upon the nature of the polymer, the polymer to drug ratio, and the relatively affinities of the drug for the polymer and the aqueous phase.[50]

As well as the drug release profile from nanoparticles can be divided into four zones: (i) initial burst period, during which the surface drug is dumped into the release medium; (ii) induction period, during which the drug is released at a gradually decreasing fast rate; (iii) slow release period, during which the drug is released at a steady slow rate; (iv) final release period (not shown), during which the particle disintegrates to release the remaining drug at a fast rate.[62] The rate of drug release from these nanospheres into buffer solution appeared to be dependant upon a number of factors including differences in EE, drug solubility, and hydrophilic nature of the copolymer.[50]

Effect of HEMA Content. It was noted from Figures 5–7 that *in vitro* release profiles for each of sodium warfarin, ibuprofen, and praziquantel from the copolymeric nanoparticles during both phases shows dependence upon the nature of the polymer in the nanospheres. The rate of drug release from copolymeric nanoparticles with monomer feed composition as MMA/HEMA50/50 was consistently faster than from copolymer that with monomer feed composition as MMA/HEMA70/30 and 90/10. The most provided faster release rates may be due to the greater hydrophilic nature of polymer MMA/HEMA50/50, with the presence of an extra hydroxyl group in every repeating unit,[63] compared to polymer MMA/HEMA70/30 and 90/10. A more hydrophilic copolymer will result in a faster ingress of aqueous fluid and so produce a faster rate of drug dissolution.[50] On the other hand, the polymer with monomer composition MMA/HEMA 90/10 shows the slowest release rates in both phases.

Effect of pH of Dissolution Media. Generally, drug solubility in various pH of the surrounding media appeared to play a dominant role in controlling release, where the release rates reached a maximum value in the simulated intestine fluid with pH 7.4, whereas released minute amounts in the gastric fluid with low pH (1.2); therefore, the copolymer was expected to release a very little amount of drug in the stomach. The three applied drugs are more soluble in intestinal fluids (pH 7.4)

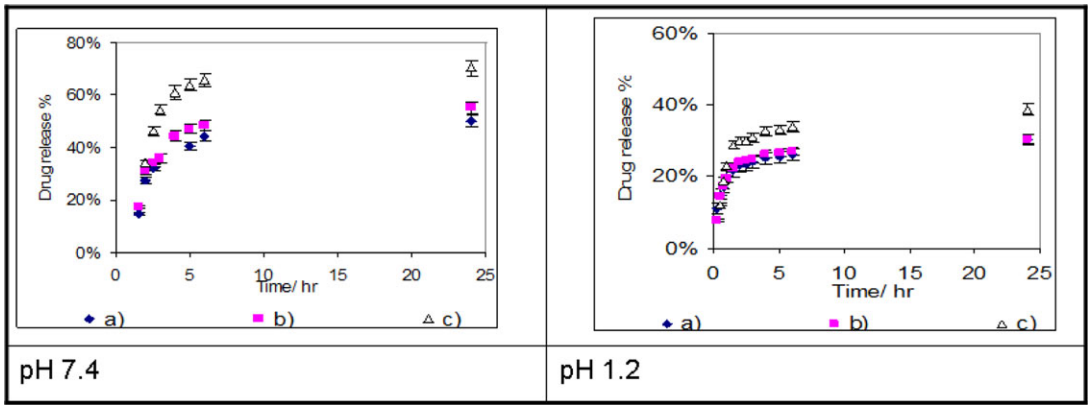

Figure 5. Release profile of sodium warfarin from the copolymeric nanosphere system in simulated intestinal fluid (pH 7.4) and simulated gastric fluid (pH 1.2) at 37 ± 0.5°C and their variation with HEMA content with monomer feed composition as: (a) MMA/HEMA 90/10, (b) MMA/HEMA 70/30, and (c) MMA/HEMA 50/50. (±S.D., n = 3). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

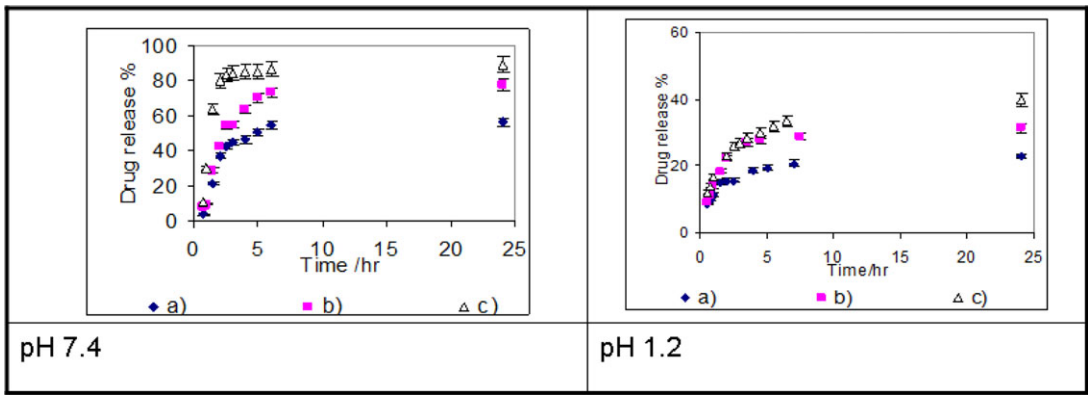

Figure 6. Release profile of Ibuprofen from the copolymeric nanosphere system in simulated intestinal fluid (pH 7.4) and simulated gastric fluid (pH 1.2) at 37 ± 0.5°C and their variation with HEMA content with monomer feed composition as: (a) MMA/HEMA 90/10, (b) MMA/HEMA 70/30, and (c) MMA/HEMA 50/50. (±S.D., $n = 3$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

than in gastric fluids, although both EE and microsphere morphology are also contributing factors.

Overall drug release profiles, either in simulated intestinal fluid (pH 7.4) or in simulated gastric fluid (pH 1.2), show a rapid initial release or burst occurred mainly attributed to weakly bound or adsorbed drug to the large surface of nanoparticles.[61,64] The degree of burst release will generally depend upon the nature of the polymer[65] and the relatively affinities of the drug for the polymer and the aqueous phase.[50] Therefore, Figures 8–10 show an extremely higher initial burst drug release into phosphate buffer (pH 7.4) than that into the gastric fluid (pH 1.2) and it can be concluded that the level of drug loading was the main factor that controlled the extent of burst release. Other possible causes of burst release included the heterogeneous distribution of drug within the nanospheres.

Figure 8 shows the release rates for sodium warfarin, in the gastric fluid which is around 23% after 4 h, whereas in the intestine fluid reached to 60% after 4 h for the most hydrophilic copolymer with monomer composition MMA/HEMA50/50. Also, Figure 9 shows very slow release of ibuprofen in buffer (pH 1.2), as it reached to 15.5–20% from MMA/HEMA 90/10-MMA/HEMA 50/50, respectively, in 2 h, while reached to 85.5% after 4 h in the intestinal phase. These data coincide with what is described in the literature.

It is noted that the release rate for PZQ (Figure 10) in the gastric fluid exceeds both sodium warfarin and ibuprofen, where the drug release rate for the copolymer with monomer composition as MMA/HEMA50/50 reaches to 45% of total PZQ after 4 h. However, in the intestinal buffer, the release of praziquantel reaches to 80.5% of total PZQ after 4 h.

Nevertheless, compared among these release profiles, it was noted that the difference in drug release profiles was the biggest for the most hydrophilic polymeric nanospheres with a monomer composition of MMA/HEMA50/50, and the smallest for the least hydrophilic polymeric nanospheres with a monomer composition of MMA/HEMA90/10. This assures that the effect of the pH of the media on drug release is also dependant on the polymer composition.

Effect of Drug Content. The rate of drug release with three different drug loadings was studied. The investigated three

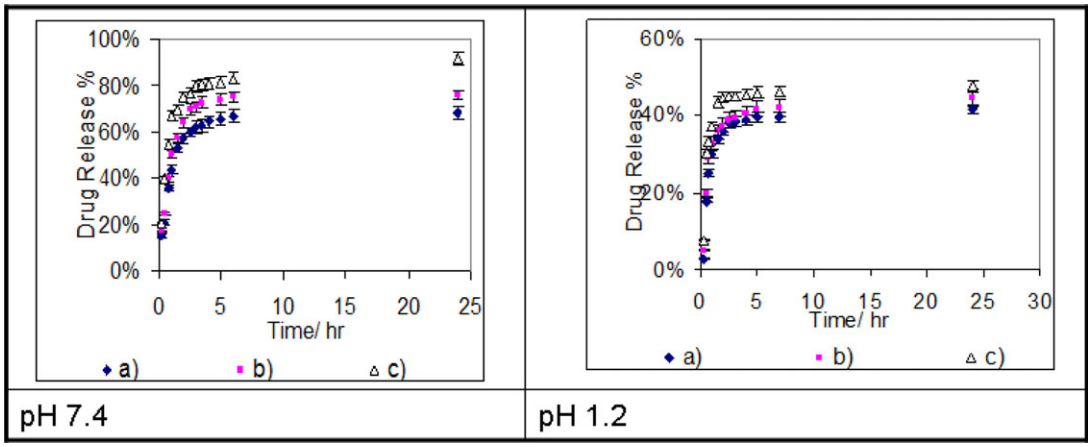

Figure 7. Release profile of Praziquantel from the copolymeric nanosphere system in simulated intestinal fluid (pH 7.4) and simulated gastric fluid (pH 1.2) at 37 ± 0.5°C and their variation with HEMA content with monomer feed composition as: (a) MMA/HEMA 90/10, (b) MMA/HEMA 70/30, and (c) MMA/HEMA 50/50. (±S.D., $n = 3$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

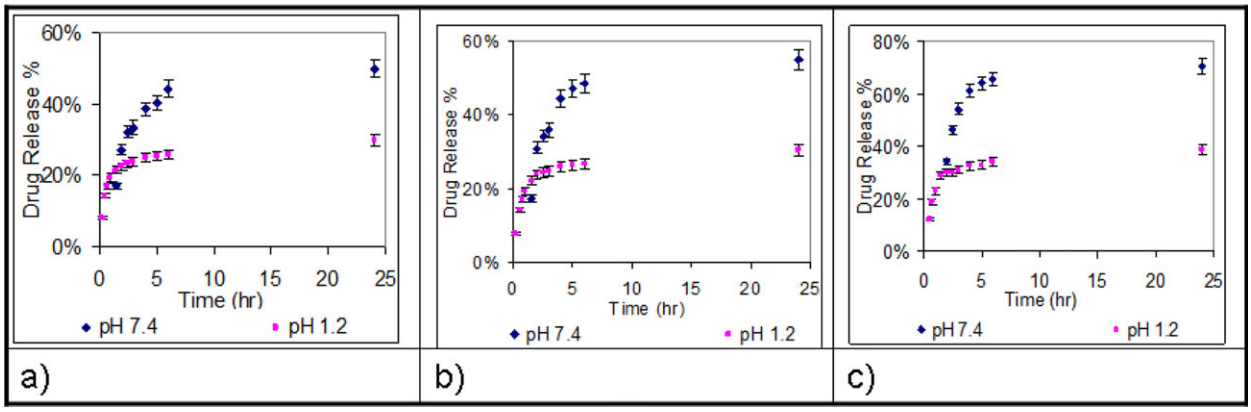

Figure 8. Effect of pH media on drug release from sodium warfarin-loaded copolymeric nanospheres with monomer feed composition as: (a)MMA/HEMA 90/10, (b) MMA/HEMA70/30, and (c) MMA/HEMA 50/50 (±S.D., $n = 3$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

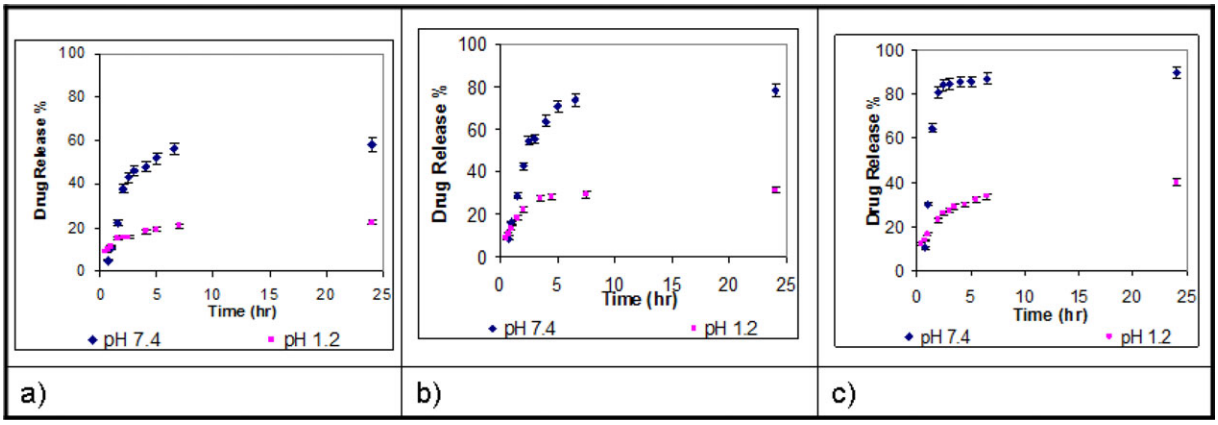

Figure 9. Effect of pH media on drug release from ibuprofen-loaded copolymeric nanospheres with monomer feed composition as: (a)MMA/HEMA90/10, (b) MMA/HEMA70/30, and (c) MMA/HEMA 50/50 (±S.D., $n = 3$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

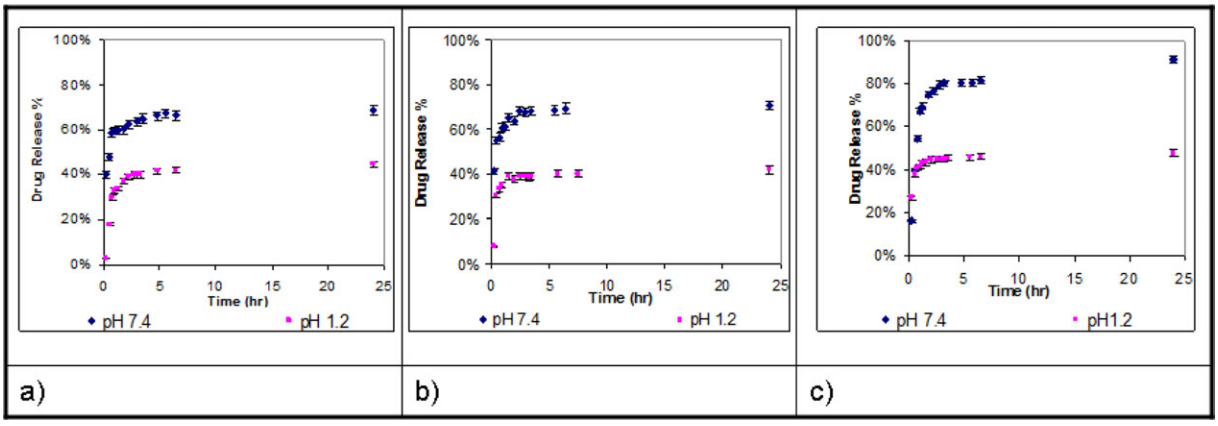

Figure 10. Effect of pH media on drug release of praziquantel-loaded copolymeric nanospheres with monomer feed composition as: (a)MMA/HEMA90/10, (b) MMA/HEMA70/30, and (c) MMA/HEMA 50/50. (±S.D., $n = 3$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

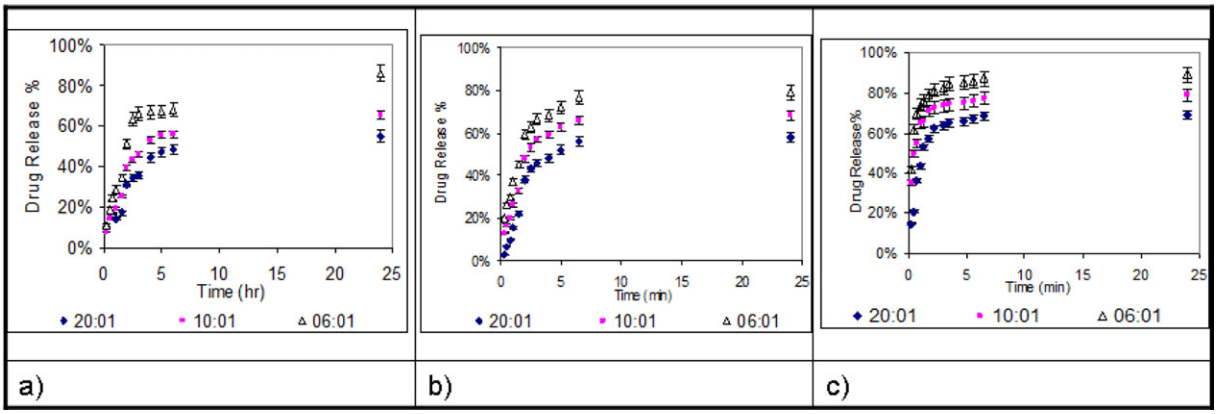

Figure 11. Effect of drug loading on drug release for (a) sodium warfarin-loaded (b) ibuprofen-loaded, and (c) praziquantel-loaded polymeric nanospheres with the same monomer composition (±S.D., $n = 3$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

different drug contents are monomer to drug ratios 20 : 1, 10 : 1, and 6 : 1 for the polymeric nanospheres with monomer feed composition MMA/HEMA90/10.

The drug release rates from the nanoparticles were tested in the simulated intestinal fluid with pH 7.4. Generally, it was noted that the release profiles were related strongly to drug content in the polymeric nanospheres.

One notes in Figure 11, that the rate of drug release from the copolymeric nanospheres is faster for the copolymer with higher drug content compared to that with less drug content. The behavior of release can be attributed to that a higher drug loading indicated a lower content of polymer matrix in the nanospheres, facilitating drug dissolution from the nanospheres.[66]

CONCLUSIONS

The utilization of well-defined copolymeric nanoparticles of methyl methacrylate/hydroxyethyl methacrylate (MMA/HEMA) with different monomer feed compositions as 90/10, 70/30, and 50/50 in entrapment and controlling the release of both hydrophilic (sodium warfarin) and hydrophobic (ibuprofen and praziquantel) drugs were achieved via (O/W) differential microemulsion polymerization technique using PVP/PEG as biocompatible emulsifiers. It was found that HEMA content in the monomer feed composition has a great effect on the colloidal properties of the produced latexes, where increase of HEMA content in the monomer feed composition lead to increase of Dv, turbidity, and the negative charge of zeta potential but decrease of the surface tension.

Moreover, the drug EE is affected by the content of HEMA in the monomer feed composition, the drug hydrophobicity, and the monomer to drug ratio. It is concluded that, EE into MMA/HEMA in monomer feed composition as 90/10, 70/30, and 50/50 was found to be (95.3–98)%, (84–96.9%), and (69.5–94.6)% for sodium warfarin (with high hydrophilicity) as well as, ibuprofen and praziquantel (with high hydrophobicity), respectively. The entrapment of drugs in polymeric nanoparticles is confirmed by IR- spectroscopy and TEM.

It was found that the EE is affected by the variation in the MMA/HEMA ratio, the drug type and the monomer: drug ratio, where it increased with both HEMA ratio, and drug content. As a general tendency, high EE was found for warfarin sodium and Ibuprofen and they were up to 95.3% and 85%, respectively, but for praziquantel, the EE reached to 75%. But overall batches showed high EE up to 95–98% with increasing monomer: drug ratio as 6 : 1. The rate of drug release was examined with respect to the variation in the MMA/HEMA ratio, the drug type, the physiological pH, and the monomer: drug ratio, where *in vitro* release properties have been investigated in simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 7.4) at 37 ± 0.5°C. Controlled release of drugs from copolymeric hydrogel nanosphere has been observed during *in vitro* release experiments. Generally, it was noted that the release profiles depended strongly on the type of drug, the nature of the dissolution media, and the polymer composition in addition to the drug content.

REFERENCES

1. Kreuter, J. *J. Controlled Release* **1997,** *16*, 169.
2. Ueda, M.; Iwara, A.; Kreuter, J. *J. Microencapsulat.* **1998,** *15*, 361.
3. Soppimath, K. S.; Aminabhavi, T. M.; Kulkarni, A. R.; Rudzinski, W. E. *J. Controlled Release* **2001,** *70*, 1.
4. Parker, D. K. *Am. Chem. Soc.* **2000,** *41*, 1511.
5. Rozner, S.; Shalev, D. E.; Shames, A. I.; Ottaviani, M. F.; Aserin, A.; Garti, N. *Colloids Surf. B* **2010,** *77*, 22.
6. Patel, A. R.; Velikov, K. P. *Food Sci. Technol.* **2011,** 44, 1958.
7. Spernath, A.; Aserin, A. *Adv. Colloid Interface Sci.* **2006,** 47, 128.
8. Vior, M. C. G.; Monteagudo, E.; Dicelio, L. E.; Awruch, J. *Dyes Pigments* **2011,** *91*, 208.
9. Gupta, S.; Moulik, S. *J. Pharm. Sci.* **2008,** *97*, 22.
10. Hammond, A.; Budd, P. M.; Price, C. *Prog. Colloid Polym. Sci.* **1999,** *113*, 142.
11. Chern, C. S.; Liu, C. W. *Colloid Polym. Sci.* **2000,** *278*, 821.
12. Tauer, K.; Ramírez, A. G.; López, R. G. *Comptes Rendus Chim.* **2003,** *6*, 1245.

13. Katime, I.; Arellano, J.; Schulz, P. *J. Colloid Interface Sci.* **2006,** *296*, 490.
14. Chen, J.; Zhang, Z. *Radiat. Phys. Chem.* **2007,** *76*, 852.
15. Xu, P.; Zhong, W.; Wang, H.; Tong, R.; Du, Q. *J. Colloid Polym. Sci.* **2004,** *282*, 1409.
16. Rabelero, M.; López-Cuenca, S.; Puca, M.; Mendizábal, E.; Esquena, J.; Solans, C.; López, R. G.; Puig, J. E. *Polymer* **2005,** *46*, 6182.
17. Koukiotis, C.; Sideridou, I. D. *Prog. Org. Coat.* **2008,** *63*, 116.
18. Xu, X.-J.; Gan, L. M. *Curr. Opin. Colloid Interface Sci.* **2005,** *10*, 239.
19. Norakankorn, C.; Pan, Q.; Rempel, G. L.; Kiatkamjonwong, S. *Eur. Polym. J.* **2009,** *45*, 2977.
20. Chuayjuljit, S.; Boonmahitthisud, A. *Appl. Surf. Sci.* **2010,** *256*, 7211.
21. Wang, H.; Pan, Q.; Rempel, G. L. *Eur. Polym. J.* **2011,** *47*, 973.
22. Lu, L.; Mikos, A. G. *Mater. Res. Soc. Bull.* **1996,** *21*, 28.
23. Bhawal, S.; Reddy, L. H.; Murthy, R. S. R.; Devi, S. *J. Appl. Polym. Sci.* **2004,** *92*, 402.
24. Zhang, H.; Ju, X.-J.; Xie, R.; Cheng, C.-J.; Ren, P.-W.; Chu, L.-Y. *J. Colloid Interface Sci.* **2009,** *336*, 235.
25. Uchegbu, I. F.; Schatzlein, A. G. Polymers in Drug Delivery; Taylor & Francis Group, LLC/CRC Press, **2006.**
26. Wang, L.-S.; Chow, P.-Y.; Phan, T.-T.; Lim, I. J.; Yang, Y.-Y. *Adv. Funct. Mater.* **2006,** *16*, 1171.
27. Ahmad, H.; Miah, M. A. J.; Rahman, M. M. *Colloid Polym. Sci.* **2003,** *281*, 988.
28. Arimoto, M.; Ichikawa, H.; Fukumori, Y. *Powder Technol.* **2004,** *141*, 177.
29. Vianna-Soares, C. D.; Kim, C. J.; Borenstein, M. R. *J. Porous Mater.* **2003,** *10*, 123.
30. Roninson, B. V.; Sullivan, F. M.; Borzelleca, J. F.; Schwartz, S. L. PVP, A Critical Review of the Kinetics and Toxicology of Polyvinyl pyrrolidone (Povidone); Taylor & Francis Inc publisher: Hardback, **1990.**
31. Nair, B. *Int. J. Toxicol.* **1998,** *17*, 95.
32. Hamidi, M.; Azadi, A.; Rafiei, P. *Adv. Drug Deliv. Rev.* **2008,** *60*, 1638.
33. Dobie, C. G.; Boodhoo, K. V. K. *Chem. Eng. Process.* **2010,** *49*, 901.
34. Zacchigna, M.; Di Luca, G.; Cateni, F.; Maurich, V. *Eur. J. Pharm. Sci.* **2004,** *23*, 379.
35. Sadtler, V. M.; Imbert, P.; Dellacherie, J. E. *J. Colloid Interface* **2002,** *254*, 355.
36. Xiao, X.; Wang, Y. *Colloids Surf. A* **2009,** *348*, 151.
37. Hussain, A. I.; Nasr, H. E.; El-Saadany, S. S.; El-Hamouly, S. H. *J. Dispersion Sci. Technol.* **2010,** *31*, 1278.
38. Porcel, R.; Jodar, A. B.; Cabrerizo, M. A.; Hidalgo-Alvarez, R.; Rodrıguez, A. M. *J. Colloid Interface Sci.* **2001,** *239*, 568.
39. Santosa, A. M.; Elaïssari, A.; Martinhoa, J. M. G.; Pichot, C. *Polymer* **2005,** *46*, 1181.
40. Sivakumar, M.; Rao, K. P. *J. Appl. Polym. Sci.* **2002,** *83*, 3045.
41. Chu, H.; Liu, N.; Wang, X.; Jiao, Z.; Chen, Z. *Int. J. Pharm.* **2009,** *371*, 190.
42. Marchal-Heussler, I.; Maincent, P.; Hoffman, M.; Spittler, J.; Couvreur, P. *Int. J. Pharm.* **1990,** *58*, 115.
43. Joshi, G. V.; Kevadiya, B. D.; Patel, H. A.; Bajaj, H. C.; Jasra, R. V. *Int. J. Pharm.* **2009,** *374*, 53.
44. Hua, S.; Mac, H.; Li, X.; Yang, H.; Wang, A. *Int. J. Biol. Macromol.* **2010,** *46*, 517.
45. Özer, F.; Beşkardeş, M. O.; Zareie, H.; Pişkin, E. *J. Appl. Polym. Sci.* **2001,** *82*, 237.
46. Braconnot, S.; Hoang, C.; Fessi, H.; Elaissari, A. *J. Mater. Sci. Eng. C* **2009,** *29*, 624.
47. Capek, I. *Adv. Colloid Interface Sci.* **2001,** *92*, 195.
48. Moustafa, A. B.; Sobh, R. A.; Rabie, A. M.; Nasr, H. E.; Ayoub, M. M. H. *J. Appl. Polym. Sci*, 7 JUN 2012, DOI: 10.1002/app.38059.
49. Valot, P.; Baba, M.; Nedelec, J.-M.; Sintes-Zydowicz, N. *Int. J. Pharm.* **2009,** *369*, 53.
50. Thompson, C. J.; Hansford, D.; Higgins, S.; Rostron, C.; Hutcheon, G. A.; Munday, D. L. *Int. J. Pharm.* **2007,** *329*, 53.
51. Zhang, J. X.; Li, S. H.; Li, X. D.; Li, X. H.; Zhu, K. J. *Polymer* **2009,** *50*, 1778.
52. Leo, A.; Hansch, C.; Elkins, D. *Chem. Rev.* **1971,** *71*, 525.
53. Katsikogianni, G.; Avgoustakis, K. *J. Nanosci. Nanotechnol.* **2006,** *6*, 3080.
54. Ingram, T.; Richter, U.; Mehling, T.; Smirnova, I. *Fluid Phase Equilib.* **2011,** *305*, 197.
55. Directive 98/8/EC concerning the placing of biocidal products on the market, Assessment Report (essay), Sodium warfarin, September (**2009),** Annex I, Ireland.
56. Shawahna, R.; Rahman, N. U. *DARU* **2011,** *19*, 83.
57. Scheytt, T.; Mersmann, P.; Lindstaädt, R.; Heberer, T. *Water Air Soil Pollut.* **2005,** *165*, 3.
58. Available at: www.wolframalfa.com, WolframAlpha What is the partition coefficient for praziquantel.mht. Accessed March 2012.
59. Graf, A.; Jack, K. S.; Whittaker, A. K.; Hook, S. M.; Radesa, T. *Eur. J. Pharm. Sci.* **2008,** *33*, 434.
60. Graf, A.; Rades, T.; Hook, S. M. *Eur. J. Pharm. Sci.* **2009,** *37*, 53.
61. Kumari, A.; Yadav, S. K.; Yadav, S. C. *Colloids Surf. B* **2010,** *75*, 1.
62. Budhian, A.; Siegel, S. J.; Winey, K. I. *Int. J. Pharm.* **2008,** *346*, 151.
63. Thompson, C. J.; Hansford, D.; Higgins, S. Hutcheon, G. A.; Rostron, C.; Munday, D. *J. Microencapsulat.* **2006,** *23*, 213.
64. Fresta, M.; Puglisi, G.; Giammona, G.; Cavallaro, G.; Micali, N.; Furneri, P. M. *J. Pharm. Sci.* **1995,** *84*, 895.
65. Chen, Y.; Mohanraj, V. J.; Parkin, J. E. *Lett. Peptide Sci.* **2003,** *10*, 627.
66. Li, C.; Cheng, L.; Zhang, Y.; Guo, S.; Wu, W. *Int. J. Pharm.* **2010,** *386*, 23.