Poly(2-hydroxyethylmethacrylate-graft-folic acid), synthesis, solubility enhancement, and release dynamic of folic acid

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ABSTRACT
A series of poly (2-hydroxyethylmethacrylate-graft-folic acid) (FAHEMA) systems are synthesized by grafting of folic acid (FA) into poly (2-hydroxyethylmethacrylate) via an esterification reaction. The structure of these copolymers is confirmed by NMR and CHN analyses. The thermal behavior of these materials is characterized by DSC and TGA analyses. The surface morphology of FAHEMA films before and after the release process is examined by the SEM method. The cumulative FA released in different pH media from FAHEMA materials occurred via a retro-esterification reaction at body temperature during 72 h in which the influence of the swelling degree of PHEMA, the FA content and pH media on the dynamic release is widely investigated. The results obtained revealed that the solubility of FA in water deduced from the release process is widely improved compared with literature reports. It is also revealed that the diffusion of water in different pH media through the PHEMA matrix and that of FA through FAHEMA materials perfectly obeyed the Fickian models. It was deduced from the kinetic study that the release performance is obtained with the copolymers containing initially 10 and 20 wt% of FA contents.

Introduction
The solubility of drugs in water is considered as a principal key in order to reach therapeutic plasma concentrations after oral administration, therefore, enhancement of their dissolution rates is necessary. More than 40% of newly discovered drugs are water-insoluble or poorly soluble, which present a serious challenge to the success of new drugs development. During the two last decades several literature reports dealt with such a problem. In fact, to enhance the solubility of drugs, Hancock [1] and Grau [2] used the physical reduction of the drug particles sizes, which led to increase their surface areas and/or the generation of amorphous states. Indeed, according to different investigators, the smaller the drug particles size, the higher is the solubility.[3–8] Solid dispersion method has also been employed by different investigators to increase the drug-water solubility. This technique applied on sulfathiazole [9] and chloramphenicol [10] by Sekiguchi led to promising results. Folic acid (FA) also called Folcacin is one of the important drugs containing three important active groups such as pteridine ring, para-aminobenzoique acid, and glutamic acid (Scheme 1), is a vitamin poorly water-soluble (1.6 μg mL⁻¹) at 25 °C.[11]

This medication is essential in human diet, necessary for maintenance in growth and production of new cells. This substance is also necessary and important for rapid cell division and growth periods such as pregnancy and infancy. So it is often recommended to take specific dosages of FA on daily basis. Folic acid and conjugated derivatives combine with folate receptor grafted on the surface of cancer cells are internalized to intracellular compartments to form endosomes.[12, 13] According to different researchers,[14, 15] the conjugation between folates receptors and conjugates separate in acidic media (pH 5 at 5.5) converted after dissociation to be released in the cytosol of cell surface or degrades by liposome. Biodegradable and biocompatible polymers used as drug support have received a considerable attention in the last two decades. Thermoresponsive hydrogels have recently become more attractive particularly in the biomedical domain; its use included controlled drug delivery of which the poly(2-hydroxyethylmethacrylate)(PHEMA) is a typical one.[16–18] PHEMA is a linear polymer composed of very active hydroxyster units distributed uniformly on the polymeric chain. This polymer is considered as one of the most important hydrophilic polymers, biodegradable, and nontoxic material.[19–22] In certain case, this polymer is considered as a living tissue, inertness to biological metabolisms, permeable to metabolites, resistant...
to absorption by the body and can easily be manufactured into many shapes and forms.\cite{23,24} PHEMA have been widely employed in different domains as medicine, biotechnology, chemical, food, feed, agriculture, and environment protection.\cite{25–31} Dilmì et al. \cite{32} investigated on the ibuprofen release kinetics from hydrogels based on the 2-hydroxyethylmethacrylate and chitosan as drug carrier. The data obtained revealed that the swelling process was controlled by second-order kinetics, while the ibuprofen diffusion through the polymer matrix obeyed the fickian model. Başbağ et al. \cite{33} have prepared three types of delivery polymer systems based on (2-hydroxypropyl)-β-cyclodextrin (HPβCD) and 2-hydroxyethyl methacrylate (HEMA). The resulting materials are poly(HPβCD-co-HEMA) hydrogels containing different HPβCD contents. It is observed that, in presence of HEMA, hydrogels have acquired mechanical integrity with lower equilibrium content than that of hydrogels without HEMA. Cyclosporine A (CsA) used as a drug is loaded into the HPβCD-based hydrogels using the suspension technique. It is revealed that the poly(HEMA) hydrogels have released practically the total CsA amount initially incorporated in these materials during three days of the release process, while, a long-term release is realized from HPβCD-based hydrogels. It is also observed that, for sub-conjunctival administration, regarding the loaded CsA amounts, release profiles, and mechanical integrity, the most suitable system is poly(HEMA-co-HPβCD) hydrogels containing high HPβCD content. Kumar et al. \cite{34} investigated the encapsulation of hydrophobic drug as curcumin in hydrophilic polymeric core as PHEMA using the nanoprecipitation method to improve its efficacy. The anticancer activity of curcumin-PHEMA nanoparticles system estimated in the ovarian cancer cells regression (SKOV-3) revealed that, this drug–polymer system had better tumor cells regression activity compared with that obtained using the free curcumin. The toxicity of PHEMA nanoparticles studied in zebrafish embryo model revealed that the material was biocompatible and the in vitro release study revealed that, the curcumin-loaded PHEMA nanoparticles have potential therapeutic values in cancer treatment. In this same subject, recently Sudhakar et al. \cite{35} reported an investigation on the release of this pharmacon from a 2-hydroxyethylmethacrylate copolymerized with N-vinylcaprolactam used as drug carrier and the results obtained revealed a formation of nanogels in a spherical shape, with the size of 150 nm and indicated that the nanoparticles may be used for targeted drug delivery.

Grafting the FA on PHEMA through a catalytic esterification reaction to prepare a new smart material capable to release uniformly adequate amount of folic acid as long as possible by retro-esterification reaction can be a solution to resolve the inconvenience associated with the drug release technique. Among the advantages of this method are to achieve optimum concentrations, prolong time, reduce the side effects, enhance the drug activity and control the dynamic release – pH relation. In this investigation a series of poly(HEMA-graft-FA) (FAHEMA) is synthesized via an esterification reaction and characterized by different methods such as NMR, CHN, DSC, XRD, and SEM. The dynamic release of FA through a retroesterification reaction in different pH media and at different FA contents is widely investigated. The solubility of FA in the media and the diffusion behavior in the polymer matrix are also investigated.

**Experimental**

**Materials**

FA is supplied by Riyadh Pharma Company and used without purification. 2-hydroxyethylmethacrylate (HEMA) (Sigma Aldrich, 98% purity) was distilled under reduced pressure and kept at −20 °C under nitrogen atmosphere. Dimethyl sulfoxide (LOBA Chemie, purity 99%) is used without further purification. Azo-bis-isobutyronitrile (AIBN) (Aldrich, 98% purity) was purified by recrystallization in methanol.

**Preparation of PHEMA**

PHEMA is synthesized by bulk polymerization of HEMA through a free radical reaction at 70 °C during 30 min under nitrogen atmosphere using AIBN as initiator and the preparation conditions is summarized in Table 1.
Preparation of FAHEMA

FAHEMA copolymer is synthesized through a catalytic esterification reaction between PHEMA and FA according to Scheme 2. A solution containing a well-defined FA amount in DMSO is added dropwise to the precedent reactor containing the resulted polymeric solution at the end of the polymerization of HEMA. The esterification reaction of PHEMA with FA carried out at 60 °C in presence of 0.5 ml of concentrated sulfuric acid. Water produced during the esterification reaction is removed by evaporation under reduced pressure using a vacuum pump. In this case, the equilibrated reaction would be forced to produce only the ester. A thick and very viscous solution is obtained from this reaction indicating that the poly (PHEMA-graft-FA) copolymer is produced. Residual FA is removed by washing the copolymer obtained several times with DMSO until the washing solution became colorless. The as prepared polymer and copolymers is left at ambient temperature for 24 h then dried under vacuum at 80 °C in a vacuum for 2 h. Noting that, the polymer and copolymers specimens heated at this temperature become water-insoluble, however, it swells in the media leading to a hydrogel – forming. A series of FAHEMA copolymers containing 5, 10, 15, and 20 wt% FA contents are prepared by the same method and their preparation conditions are given in Table 1.

Equipment and methods

The average number molecular weights of the prepared PHEMA and FAHEMA with different FA contents were estimated in DMSO at 60 °C by size exclusion chromatography (SEC) on a Varian apparatus. This instrument is equipped with a JASCO-type 880-PU HPLC pump with a flow rate of 1.0 ml, refractive index and UV detectors and Shodex GPC KF-806 M (8.0 mm I.D. x 300 mm) columns calibrated with polystyrene standards and the results obtained are summarized in Table 2.

\(^1\)H NMR and \(^{13}\)C NMR analysis of FA, PHEMA, and FAHEMA copolymers containing different FA contents are performed on a JEOL FX 90 Q NMR apparatus at 500 and 200 MHz, respectively in DMSO-d\(_6\) at room temperature. The composition of 2-folatethylmethacrylate comonomer (FEMA) in FAHEMA systems are determined by CHN elemental analysis using Perkin Elmer PE 2400 Series II apparatus and the results of FAHEMA characterization are grouped in Table 2. The FA amounts released from the synthesized materials are determined by UV–Vis Aultropec 2100 pro, Amersham Biosciences spectroscopy at 281 nm using FA calibration curve. The release experiments are carried out at 37 °C in pH media of 1, 3, 5, and 7. The melting points (T\(_{m}\)) of FA and the glass transition temperatures (T\(_{g}\)) of PHEMA homopolymer and FAHEMA copolymers are performed on a DSC (Shimadsu DSC 60) apparatus, previously calibrated with indium. Samples weighing between 10 and 12 mg are packed in aluminum DSC pans before placing in DSC cell. The samples are heated from −50 to 200 °C at a heating rate of 20 °C min\(^{-1}\). For the surface morphology analysis, scanning electron microscopic (SEM) micrographs of dried thin films of FA, PHEMA, and FAHEMA films coated with gold grid are obtained using JSM-6060LV (JEOL). X-ray measurements of FA, PHEMA, and FAHEMA copolymers with different FA contents are collected from a BRUKER D8 advance diffractometer (Germany). The patterns were run with Cu K\(_\alpha\) radiation at 40 mA and 40 kV with scanning speed in 2\(\theta\) of 2° min\(^{-1}\).

The cytotoxicity test of pure PHEMA and HEMAFA materials is realized by MITT assay on L929 fibroblastic cell lines for cell toxicity evaluation according to Mosmann method. [36] These cells are placed in Dulbecco’s modified Eagle’s medium (DMEM) complemented with 10% of fetal bovine serum (FBS) placed in an incubator maintained in a humidified atmosphere containing 5 wt% of CO\(_2\) at body temperature. The cells are trypsinized during 4 min. The cells cultured are incubated with different concentrations of PHEMA and HEMAFA system in DMEM/FBS solution ranged

| Sample     | FA (g) | HEMA (g) | FA (wt%) | AIBN (mg) | DMSO (ml) |
|------------|--------|----------|----------|-----------|-----------|
| PHEMA      | –      | 4.00     | –        | 6.8       | 20        |
| FAHEMA5    | 0.199  | 3.790    | 3.99     | 6.8       | 20        |
| FAHEMA10   | 0.399  | 3.590    | 9.98     | 6.8       | 20        |
| FAHEMA15   | 0.590  | 3.400    | 14.76    | 6.8       | 20        |
| FAHEMA20   | 0.790  | 3.190    | 19.81    | 6.8       | 20        |
between 10.0 and 100 μg mL⁻¹ for 24 h. The cells obtained from this step are then washed with PBS and stained with 50 μL of MITT solution. After 4 h, the medium is separated and the solution is measured at 570 nm using a microplate reader (Statfax 2100). The percentages of viability and toxicity are evaluated by Equation (1) and Equation (2), respectively [37]

\[
\text{Viability (\%)} = \frac{D_T}{D_C} \times 100
\]

in which the toxicity is deducted from the following relationship:

\[
\text{Toxicity (\%)} = 100 - \text{Viability (\%)}
\]

where \(D_T\) and \(D_C\) are the optical density of test and that of control, respectively.

**In vitro drug release**

FAHEMA film samples were immersed in defined pH media for 2 h which corresponded to the maximum swelling of PHEMA and dissolution of any FA traces deposited on the surface. The effective FA/PHEMA composition is obtained from a simple subtraction of the FA amount dissolved during the washing time (2 h) from that initially incorporated in the PHEMA matrix. FAHEMA films are placed in 15 ml of water/hydrochloric acid solution at a fixed pH and stirred at 130 rpm and 37 °C (body temperature). This experimentation is performed at pH 1, 3, 5, and 7. The film sample immersed in defined pH media is taken to a maximum swelling and dissolution of any FA traces deposited on the surface. Noting that the time and the FA amount dissolved in the media during this step is not taken in consideration in the cumulative FA-released calculation and the real FA/PHEMA composition was corrected by a simple subtraction of the FA amount dissolved from that initially incorporated in the PHEMA matrix. Aliquots of 0.5 ml are then withdrawn at time intervals. To prevent the concentration equilibrium between FA in the polymer matrix and FA in the media, the solution is immediately changed after each measurement. This operation is kept at constant volume of media and ensured a continuous release of FA during all the release process. The total mass of FA released during a definite duration is calculated from the standard absorbance curve obtained by UV analysis as mentioned above. It is important to note that during the release process the pH of water is practically not affected by the small amount of FA released because the amount of FA released is negligible compared to those of the media, however, FA is a weak acid \(pK_{a1} = 4.7\), therefore the addition of a buffer solution to water is not necessary. It is important to note that no polymer or other substances which can be produced during the release process were deposed on the bottom of the reactor except the FA released which its structure was proved by ¹HNMR and its melting temperature by DSC analysis.

**Results and discussion**

**Characterization**

The poly (HEMA-graft-FA) structure is confirmed by ¹HNMR analysis through the comparison of the FAHEMA spectrum with those of pure PHEMA and FA presented in Figure 1. Indeed, in addition to the absorption signals attributed to both FA and PHEMA observed in the FAHEMA spectra, the intensity reduction of the signal (e) localized at 4.70 ppm is attributed to the hydroxyl proton assigned to the 2-hydroxyethylmethacrylate unit, while an increase in the signal at 3.81 ppm was attributed to the four protons...
(c + c') of the two ester methylene groups (−CO−O−CH₂−) of the 2-folateethylmethacrylate ester unit.

The comparison of the FAHEMA ¹³C NMR spectra with those of the pure PHEMA and FA as shown for FAHEMA20 in Figure 2 indicates the presence of HEMA unit and FA substituent in the copolymer synthesized. The structure of FAHEMA material is confirmed by the increase of the peak localized at 61.21 ppm assigned to the carbon (d) linked to the oxygen ester (−CH₂−O−CO−) of the folate substituent and also by the intensity decrease in the peak at 57.3 ppm attributed to the carbon (e) directly linked to the hydroxyl group (HO−CH₂−) of HEMA unit in the copolymer.

The molar fraction of 2-folateethylmethacrylate comonomer (FEMA) in the copolymers, determined previously by CHN analysis, is confirmed by ¹H NMR using the surface area of the two equivalent ethenyl protons (11), δCH₂ in FEMA structure centered at 2.27 ppm and those of the 3 protons (a) of the methyl group, δCH₃, common to the two comonomers localized between 0.40 and 1.00 ppm using Equation (3).

\[ \chi_{\text{FEMA}} = \frac{\delta_{\text{CH}_2}}{3 \times \delta_{\text{CH}_3}} \] (3)

The results of the molar fractions of FA obtained from the ¹H NMR data and Equation (3) grouped in Table 2 perfectly agreed with those previously obtained by the CHN method.

The DSC thermograms of PHEMA, FA, and FAHEMA with different FA contents are gathered in Figure 3. On the light of these curve profiles, a shift in the \( T_g \) value towards the high temperatures (from 50 to 57 °C) is observed in the thermograms of FAHEMA copolymers compared with that of the corresponding homopolymer. The increase in \( T_g \) is probably caused by a reduction of the chains sliding due to the sterically hindered folate grafted on PHEMA chains. As shown by the thermogram of FAHEMA containing 20 wt%
of FA content, the transition showed at 110 °C is probably attributed to a shift in the melting temperature of the residual free FA aggregated and homogeneously distributed in the copolymer matrix. Noting that the results obtained by \(^1\)H and \(^{13}\)C NMR confirmed the presence of a small amount of FA none grafted incorporated in the copolymer materials. The shift in the glass transition temperature towards the right is probably caused by the inter-chains reaction between some FAHEMA chains in acidic media during the preparation process. Indeed, this fact assists the formation of a weakly cross-linked polymer leading to a reduction in the chains slide and consequently leads to an increase in the \(T_g\) value. However, the shift in the melting temperature attributed to the free FA aggregated into the copolymer matrix is generally caused by the slow heat transfer into the polymer material.

XRD analysis performed on the pure PHEMA, FA, and FAHEMA with different FA contents have led to the spectra of Figure 4. The XRD pattern of FA revealed a multitude of absorption peaks in which the principals are centered at 5.5, 11.0, 13.5, 22.0, 27.0, and 29.0 two-theta characterizing its crystalline structure, which perfectly agreed with those of the literature.\(^{[38,39]}\) While that of PHEMA is devoid of any signal indicating a crystalline structure. The FAHEMA

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**Figure 2.** \(^{13}\)C NMR spectra of pure FA, PHEMA, and FAHEMA20 in DMSO-d6.
samples with different FA contents revealed in their spectra a total disappearance of the peaks attributed to the crystalline structure of FA. However, new signals in which the principal peaks appeared at 34.0, 41.0, 48.0, 53.5, and 54.5 two-theta confirm the presence of free FA aggregated in another crystalline system distributed uniformly in the copolymer matrix. Indeed, folic acid has an extended conformation caused by the pteridine ring in which the equilibrium between the ketonic and enolic forms (Scheme 1) can lead to different crystalline systems.

The cytotoxicity activity of the FAHEMA drug-carrier system with different FA content is presented in Figure 5. As it can be seen from these diagrams which show a viability superior to 68% for all concentrations range (0.010–0.001 g ml⁻¹) indicating that all specimens are out of the toxic level.[40,41] These results also reveal that the percentage of viability slowly decreases as the FA content grafted in the PHEMA increases and the best viability was observed at concentration of 0.010 g ml⁻¹.

**Swelling behavior of PHEMA**

The swelling study of a polymer hydrogel in water is indispensable to understanding the diffusion of drug-water solution through a material used as carrier in the drug delivery domain. Indeed, Wan et al. [42] who used the hydroxypropylmethylcellulose (HPMC) as a hydrophilic drug support revealed that an inverse relationship exists between the drug release rate and matrix swelling rate. The swelling behavior of HPMC is therefore useful in predicting drug release. In this investigation, a dried PHEMA film was immersed in defined pH media maintained at 37 °C under stirring for 4 h corresponding to the maximum swelling of PHEMA. Noting that, at the end of the swelling process, no traces of polymer were observed after removing the specimen and evaporation of water (media). This preliminary test indicates that this polymer is not soluble in water. However, it swells in the media leading to a hydrogel – forming. The capability test of PHEMA hydrogel to swell in different pH media evaluated using Equation (4).

\[
S(\%) = \frac{w_t - w_o}{w_o} \times 100
\]

where \(w_o\) and \(w_t\) are the weight of the film sample before and at \(t\) time of the swelling process, respectively. \(S(\%)\) is the swelling degree of PHEMA in different media. Noting that after specimen withdrawing and water (media) evaporation no residual polymer was observed at the bottom of the beaker.

On the light of the variation of the swelling degree vs. time presented in Figure 6 it is revealed that the maximum swelling, \(S_\infty\) corresponding to the equilibrium is practically reached at about the two first hours of the swelling process.
immersed into a penetrating media during a determined time, \( t \). According to Comyn [43] the kinetics that governs this dynamic is generally given by Equation (5).

\[
\frac{M_t}{M_\infty} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2\pi^2} \exp \left[-\frac{D(2n+1)^2\pi^2t}{l^2}\right]
\]

(5)

where \( M_t \) and \( M_\infty \) are the mass of the absorbed molecules during \( t \) time and at the maximum absorption (equilibrium), respectively. \( l \) and \( D \) are the film thickness and the diffusion coefficient, respectively. For the short times of the initial stage of diffusion and when the \( M_t/M_\infty \) ratio is lower than 0.5 Equation (4) takes the following Equation (6) in which \( D \) can be deduced from the slope of the linear portion of the curve corresponding to the variation of \( M_t/M_\infty \) vs. square root of time. For the water/PHEMA system, Figure 7 reveals straight lines during the first hour of the swelling process indicating that the diffusion of water molecules through the PHEMA matrix obeys the fick's model as long as the temperature of this polymer in media (37 °C) is well above \( T_g \) (50 °C). This finding indicates that the diffusion of water through the polymer film is purely and simply governed by a mechanical process and non-disturbed by a probable esterification reaction which can occur between the alcoholic polymer and acidic media.

\[
\frac{M_t}{M_\infty} = 2 \times \left(\frac{D \times t}{\pi \times l^2}\right)^{1/2}
\]

(6)

and the performance of PHEMA to swell in water at 37 °C is observed at pH 3 in which 49.11 ± 0.30 wt% of water is absorbed. However, only 37.59 ± 0.27 wt% is reached at pH 1 as shown in Table 3.

The swelling kinetics is usually used to investigate the diffusion of small molecules through polymeric materials and the performance of PHEMA to swell in water at 37 °C is observed at pH 3 in which 49.11 ± 0.30 wt% of water is absorbed. However, only 37.59 ± 0.27 wt% is reached at pH 1 as shown in Table 3.

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Table 3. Swelling properties of water-PHEMA system taken at different pH media and 37 °C.

| \( S_\infty \) (wt%) | pH   | \( n \) | \( k \times 10^2 \) (s\(^{-0.5}\)) | \( D \times 10^8 \) (cm\(^2\) s\(^{-1}\)) |
|-------------------|------|------|-----------------|-----------------|
| 42.54             | 42.54| 0.5  | 4.19            | 63.67 \times 10^{-8} |
| 49.11             | 49.11| 0.5  | 3.67            | 62.48 \times 10^{-8} |
| 37.59             | 37.59| 0.5  | 3.45            | 54.87 \times 10^{-8} |
| 39.58             | 39.58| 0.5  | 3.38            | 52.96 \times 10^{-8} |

Figure 5. Cytotoxicity study of FAHEMA drug-carrier system with different FA contents using the MTT assay.

Figure 6. Variation of the swelling degree in different pH media vs. time.

Figure 7. Variation of the cumulative water absorbed, \( M_t/M_\infty \), vs. square root of time.
According to Massaro et al. [44] the basic equation of mass uptake by polymer material is given by Equation (7).

\[
\frac{M_t}{M_\infty} = k \times t^n
\]  

(7)

where \(n\) exponent is the type of diffusion mechanism and \(k\) is the constant which depends on diffusion coefficient and film thickness. By analogy with equation (3) \(n = 0.5\) and \(k\) takes the following expression (8)

\[
k = 2 \left( \frac{D}{\pi} \right)^{0.5}
\]  

(8)

As it can be seen from the \(D\) and \(k\) values reported in Table 3 that the diffusion rate slightly decreased as the pH media increased, thus indicating a very low influence of the pH-media on the swelling process.

### Solubility enhancement of FA in water

Solubility of FA in water as any other therapeutically bio-active substance is also considered as a key property in which this crucial parameter governs its dissolution in the media then its absorption by different organs. For this reason the in vivo FA efficacy depends on its solubility. FA is a crystalline drug, very slightly soluble in water (1.6 mg L\(^{-1}\)) at 25 °C [13] and its solubility varies with temperature and pH media.[45–47] Hence, poorly absorbed from the gastrointestinal (GI) tract when used alone.[48,49] Therefore, to enhance the solubility of FA in water constitutes one of our important objectives. Indeed, according to different investigations, the smaller the FA particle size, the higher the solubility.[3–8] In this work, the maximum concentration of FA is deduced from the FA released at 37 °C in different pH media during one week. Noting that, at the end of the release process, only FA was deposited in the bottom of the Becker after removing the specimen and total evaporation of media. The \(^1\)HNMR and DSC analyses confirmed this observation through a perfect superposition of their spectra and thermograms with those of the starting FA. The data of the FA-water solubility gathered in Table 4 reveals that the solubility of FA in the media increased with the FA content grafted, and the higher solubility is reached with FAHEMA20 in which more than 272 mg L\(^{-1}\) of FA is completely dissolved at pH 7 and more than 343 mg L\(^{-1}\) dissolved at pH 1.

From these observations, it is possible to conclude that the solubility of FA in different pH media has easily gone over these values, because no precipitate is observed during this period indicating that the saturation of the FA solutions is far from being reached. From this finding, it is important to emphasize that the incorporation of FA in PHEMA by grafting through an esterification reaction could be an appropriate method to increase the solubility of FA in water without addition of a third component or using the physical techniques such as the comminution or micronization techniques based on the mechanical stress to disaggregate the active compound inducing the drug degradation which is caused by the thermal and mechanical forces such as milling and grinding.[50–54] This finding is predicted because the FA released via a retro-esterification reaction is completely dispersed in water in its molecular form and not in aggregate or crystalline form. In this state, the maximum hydration of FA molecules is reached leading to the maximum solubility compared with that of Zhang et al. [55] in which the addition of a low generation polyamidoamine dendrimers enhanced a maximum solubility to 200 mg L\(^{-1}\) at pH 5. Noting that, the folic acid in suspension investigated by Pardeike et al. [39] revealed a saturation solubility of 8.96 mg L\(^{-1}\). Reducing the particle size of folic acid to the nanometer range by the same investigators led to an increase in the saturation solubility only to 13.77 mg L\(^{-1}\). It could be highlighted that the US Department of Health and Human Services announced a rule that the folic acid should be added to grain products as a nutrition fortifier to ensure 0.4 mg intake for an adult and an additional 0.2 mg for pregnant women.[56,57]

### In vitro study of folic acid released

The dynamic release of FA from poly(HEMA-graft-FA) copolymers films with 5, 10, 15, and 20 wt% of FA contents is investigated during 72 h at media of 1, 3, 5, and 7 pH at body temperature. The cumulative folic acid released during time is calculated from Equation (9)

\[
R(\text{wt%}) = \frac{m_t}{m_0} \times 100
\]  

(9)

where \(m_t\) and \(m_0\) are the mass of FA released in the media during \(t\) time and at \(t = 0\), respectively. The results obtained from this equation and the experimental data are presented by Figure 8. As it can be seen from the curves profiles of the pretreated specimens the percentage of FA released vs. time follows a perfect logarithmic growth except that of FAHEMA20 in which the dynamic release in media pH 7 evolves following a perfect straight line. As it can be seen from these data the higher dynamic release

| System      | pH media | FA amount dissolved (mg L\(^{-1}\)) |
|-------------|----------|-----------------------------------|
|             |          | 1       | 3       | 5       | 7       |
| FAHEMA5     | 199.0 ± 2.6 | 212.7 ± 3.2 | 204.1 ± 3.4 | 180.4 ± 4.6 |
| FAHEMA10    | 204.0 ± 3.2 | 217.6 ± 3.0 | 233.8 ± 3.1 | 214.7 ± 3.2 |
| FAHEMA15    | 287.4 ± 3.8 | 287.0 ± 3.0 | 267.4 ± 2.8 | 222.3 ± 3.0 |
| FAHEMA20    | 349.2 ± 2.6 | 292.2 ± 3.0 | 278.2 ± 3.6 | 272.3 ± 3.5 |
is reached at pH 7 for all FAHEMA specimens during 72 h except that containing 5 wt% of FA content in which the maximum cumulative FA released is reached in pH 5. On the other hand, for all FAHEMA compositions, the lower dynamic release is observed in media pH 3 except that with 20 wt% of FA which shows a minimum cumulative FA released in media pH 1.

The comparison of the results obtained using the different FAHEMA specimens reveals that the higher cumulative FA released is obtained using the FAHEMA containing 5 and 10 wt% FA content in which more than 60 and 68 wt% of folic acid are released in media pH 5 and 7 during 72 h of the release process, respectively. This finding reflects the capacity of the media to dissolve this drug poorly soluble in water, notably when is entrapped in the PHEMA matrix. Indeed, at any pH media, for a same mass of FAHEMA an amount of 5 wt% of FA released is easily dissolved in water than 20 wt%. SEM micrographs of FAHEMA10 film taken as example before and after the release in pH media 1 and 7 are gathered in Figure 9. The PHEMA and FAHEMA10 images obtained before the release process show a homogeneous and a smooth surface morphology. Whereas, the images of the specimen containing 10 wt% of FA obtained after the release process showed dense interconnected deep microgrooves less than 0.2 μm in width randomly oriented and homogeneously distributed in the polymer matrix. The density and the size of these microgrooves are slightly more marked at neutral pH, thus justifying the high FA amount released in these conditions.

**Diffusion behavior of FA through FAHEMA films**

According to Lin et al. [58], for a release less than 60 wt% of the initial amount loaded, the dynamic release followed the Fickian model for the diffusion from a polymeric film. The value of the diffusion coefficient, $D$, can be calculated according to the following Equation (10).\[59–62\]

$$D = \frac{0.196 \times l^2}{t} \left( \frac{m_t}{m_o} \right)^2 \tag{10}$$

where $l$ is the thickness of the film and $m_t$, $m_o$, and $t$ are as defined previously.
Effect of the swelling degree

The variation of the cumulative FA released from FAHEMA materials taken at 10 h of the release process vs. the swelling degree of the PHEMA matrix is shown in Figure 11. It was revealed from the curves profiles obtained a maximum rate of FA released when the swelling degree is 39.58 wt%, then dramatically decreased and stabilized beyond. The magnitude of this summit which is common for all FA/PHEMA compositions decreased as the total FA amount in the PHEMA matrix increased. This finding revealed that the optimum conditions to obtain performing release is reached with a material containing the lower FA content (5 wt%) and a PHEMA carrier capable to swell with 39.58 wt% and not with that with the higher swelling degree (49.11 wt%). Based on the principle that stipulates a higher performance is reached with a polymer carrier characterized by higher swelling parameters (Table 4) are not sufficient in this case. Contrarily an inverse relationship is revealed between the FA release rate and the PHEMA film...
Effect of the FA content in FAHEMA system

The influence of the total drug content grafted on PHEMA on the FA released is carried out at different pH media during 10 and 24 h of the release process which corresponds to the average intestinal transit time (AITT) [54–65] and the results obtained are gathered in Figure 12. As it can be seen from these curve profiles that at any pH medium and at any duration the variation of the FA released vs. the total FA grafted on PHEMA increased and reached a minimum at about 15 wt% of FA content, notably those at pH 5 and 7 during 24 h of the release process, except that at pH 1 in which the dynamic release continue to decrease slowly.

The dramatic decrease in the cumulative FA released could be explained by an eventual reduction of the swelling degree of the polymer matrix due to the reduction of the hydrophilicity of FAHEMA caused by an increase in the ester groups when the FA content increased. The increase in FA released from the samples containing more than 15 wt% of FA content is probably due to a supplementary dissolution of a residual FA amount (non reacted) trapped initially in the polymeric matrix.

Figure 10. Variation of the diffusion coefficient \( (D) \) of FA through the FAHEMA film with different FA contents vs. \( t^{-1} \) at different and pH media.

Figure 11. Variation of the cumulative FA released from FAHEMA systems vs. the swelling degree of PHEMA at equilibrium.
to the decrease in the solubility of the free FA initially entrapped in the copolymer matrix, bearing in mind that the solubility of FA increased as the pH media decreased in the acidic range. Whereas, the cumulative FA released from FAHEMA20 which contained the highest rate of FA grafted evolved with the pH media following a totally different behavior compared with those containing an inferior FA content. The percentage of FA amount released from this material passed through a maximum at pH 3 then decreased beyond. The sudden drop of the FA released at pH above 3 is probably due to the decrease in the swelling degree of the material due to the decrease in its hydrophylicity caused by the increase in the folic ester group substituted. At pH 1 in which the esterification reaction is favored. The cumulative FA released was minimal and results mostly by the dissolution of the residual free FA entrapped in polymer matrix.

**Kinetic study of released FA**

The instantaneous release rates of FA liberated from FAHEMA systems are determined from the slopes of the
linear portions of the curves indicating the variation of the cumulative FA released vs. time of Figure 8 and the results obtained are gathered in Table 5. From these data, it was revealed in all samples, the presence of two principal stable zones of the release rate except that of FAHEMA containing 20 wt% of FA in which only a single zone was observed at pH 1 and 7. The first one is relatively short; varying between 6 and 28 h depending on the initial FA content grafted on PHEMA and the pH media is characterized by a relatively high release rate which is probably due to the rapid dissolution of the first free FA molecules liberated from the retro-esterification reaction of the external macromolecules on the surface of the specimens.

This phenomenon is also reported by different authors using other drug-carrier systems as poly (lactic acid)/drugs composites by Taranti et al. [66], and by Zilberman [67] using Dexamethasone-loaded biore sorbable films which attributed the first zone to different reasons: (i) drug particles embedded on the external surface of granules due to imperfect encapsulation and/or (ii) the solubility. The second zone is relatively long and slower, varying between 32 and 65 h also depending on the initial FA content grafted on PHEMA and the pH media is attributed to the FA released inside the specimens according to a mechanism based on a retro-esterification reaction of FAHEMA copolymer followed by a diffusion of the released drug through the polymer matrix. On the other hand, as mentioned previously, FAHEMA 20 which contained 20 wt% of FA presents an ultimate stable zone at pH 1 and 7 throughout the lifespan of the release process. Basing on the criteria, higher cumulative FA released in neutral pH, longer time, adequate release rate, it was revealed that the FAHEMA containing 10 wt% of FA grafted has the best performance. Indeed, this specimen is capable to release the higher cumulative FA (54 wt %) at neutral pH uniformly with a release rate of 0.92 during the longer time (59 h). On the other hand, this same specimen released only 24 wt% of FA at pH 1 with a release rate of 0.33 wt%/h during practically the same period. The second position is occupied by FAHEMA containing 20 wt% of FA which is capable to release uniformly 47.0 wt% of FA with a release rate of 0.65 wt%/h during 72 h. However, this same material released only 10 wt% of FA uniformly with a release rate of 0.15 wt%/h during practically the same time.

**Conclusions**

It could be concluded from this investigation that the incorporation of drugs containing carboxylic groups such as FA that is slightly soluble in water by grafting on the polymer containing reactive hydroxyl groups via an esterification reaction could be an appropriate method that can be used in drug delivery domain. Indeed, this method leads to the best distribution of FA in its molecular state in the polymer matrix and by this way considerably increases the water–drug contact and widely favored its solubility in the media. Indeed, the solubility test of FA in water estimated from the maximum release of FA indicates that the FAHEMA20 containing the higher FA grafted easily exceed 272 mg L⁻¹ at neutral pH and 343 mg L⁻¹ at pH 1 a rate that is never reached by the traditional methods. The diffusion of water through PHEMA matrix and that of FA through FAHEMA system at different pHs revealed that the diffusion behavior in the stationary regime perfectly obeyed Fickian models. The higher performance of the cumulative FA released from FAHEMA systems is obtained at pH 7 with FAHEMA containing 10 and 20 wt% of FA content. This finding seemed
to be very interesting in drug release field because these systems are capable to release uniformly only a small FA amount during the stomach transit time (pH 1, duration ~2 h). However, the majority of FA amount would be uniformly released during the intestines transit time (pH 7, duration ~70 h).

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