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Synthesis and Characterization of Arabic Gum-Grafted-Polyethylene Imine Microgel

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ABSTRACT
Microgels prepared from natural and synthetic polymers continue to attract the interest of medical and pharmaceutical researchers due to their tunable properties by modifying their molecular structure through derivatization, cross-linking or grafting method. This research developed Arabic gum microgels as smart and stimuli-responsive materials for various applications. Chemically modified Arabic gum microgels were prepared by emulsion polymerization system using Tween20 as surfactant and hexane as the solvent. The modifying agent's polyethylene imine were successfully cross-linked using N, N'-methylenebisacrylamide and precipitated using sufficient acetone. Physico-chemical methods of characterization such as Fourier transform infrared, differential scanning calorimetry and X-ray diffraction were used to analyzed and confirmed the successful synthesis of the chemically modified microgels. The surface morphology of the microgels were observed using Field emission scanning electron microscope which shows the presence of network structure and porosity arrangements. In response to pH microgels of polyethylene imine swelled more in lower or acidic pH and a lower swelling ratio was observed in higher pH solutions. The swelling of hydrogels also depends on the compositions of the swelling medium. The presence of ions in the medium reduces the swelling ratio for the modified hydrogels. The results confirm the potentials of chemically modified microgels in Industrial and pharmaceuticals applications.

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INTRODUCTION

Carbohydrates polymers are the types of biopolymers that have vast application in medicine due to compatibility and the ability to degrade in an organism without causing any damage to the system (Nair *et al.*, 2006). Biopolymers are largely considered as an eco-friendly replacement to petrochemical polymers use for pharmaceutical applications due to the renewable feedstock used to produce them and their biodegradability. However, some biopolymers are lacking in some basic properties such as biocompatibility, dissolubility and mechanical activity in solutions (Oh *et al.*, 2009). Therefore, researchers are making various efforts either through surface modification, breakage of linkage or cross-linking to obtain the desired property of the biomaterial for a particular biomedical application (de Las Heras Alarcon *et al.*, 2005).

Modification is an artificial method to manipulate the properties of gum to attain a desirable physically and chemically properties. Various researchers have been explored to modify the properties of these polysaccharides through crosslinking, surface modification and breaking of the polymer chain to attain the required property for the desired application (Aamir *et al.*, 2019; Agnello *et al.*, 2017; BeMiller *et al.*, 2012; de Las Heras Alarcon et al., 2005). Microgels are polymers with particle dimension ranging from 10-1000 nm which are far smaller in size compare to

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the macrogels. Microgels response to stimuli faster compares to macrogels which response to external condition slowly depending on the chain length of the polymer, nature and type of crosslinking agent and finally the functional groups present in the hydrogel network structure (Knipe *et al.*, 2014).

The swelling and de-swelling of microgels are rapid and fast and therefore provide a larger and more exposed surface area for reaction to take place as compare to macrogels (Saunders *et al.*, 1997). For environmental concerns such as pollution caused by heavy metals and carcinogenic dyes, the microgels can be used to remove the pollutants through adsorption. Functionalization of the microgels or incorporation of metals nanoparticles inside the porous microgel matrix may catalyze the process of pollutant reduction and adsorption (Sahiner, 2013; Tsarevsky *et al.*, 2007). Industries use gel substances to enhance the properties of substances such as paint and resins, using microgels resins can also be functionalized against weathering of fabricated materials composite (Raquois *et al.*, 1995).

MATERIALS AND METHODS

Arabic gum (AG, Mw: 250,000 g/mol, Sigma Aldrich), polyethylene imine (PEI, 50% (w/v), Sigma Aldrich), N, N'-methylenebisacrylamide (MBA, Mw: 154.17 g/mol 99%, Sigma-Aldrich) and N, N, N', N'-tetramethylethylenediamine (TEMED, Mw: 116.20 g/mol, 99.5% Sigma-Aldrich). Tween 20 (T20, Polyethylene glycol sorbitan monolaurate), Chemical reagents such as methanol and ethanol (99%, Sigma-Aldrich), hexane (Mw: 86.18 g/mol 99%, Sigma-Aldrich) and sodium hydroxide pellets (Mw: 40.0 g/mol) was purchase from Friedemann Schmidt. Redox pair initiator (APS, Mw: 228.20 g/mol, Sigma-Aldrich), acetone (Mw: 58.08 g/mol 99%, Merck), while sodium chloride, aluminium chloride, calcium chloride and potassium chloride were purchased from Merck. All chemicals were used as obtained.

Formulation of Arabic gum polyethylene imine Microgels via Emulsion Polymerization

Arabic gum with polyethylene imine microgels were synthesis using emulsion system. A 10 % solution of Arabic gum in 0.5 M NaOH solution was made and stirred for one hour to form a homogenous solution. After that 10 ml of the prepared solution was transferred into 100 ml reaction bottle containing 50 ml of the emulsion system of 5 % w/v aqueous T20 in hexane. The mixture was stirred for 30 minutes at 1000 rpm, 2 ml of 2.5% ammonium persulfate solution was added and stirred for 30 minutes at 60 °C, followed by the addition of 2 ml polyethylene imine (50%w/v) solution and after 30 minutes about 100 mg N, N'-methylene bis-acrylamide (MBA) as the crosslinking agent was added to crosslink the polymers in the reaction.

The colour of the mixture changes to light yellow indicating the formation of particles through crosslinking. The stirring was continued for additional 30 minutes for complete crosslinking of the polymer particles and AG chains. The product was precipitated using an excess amount of acetone, the emulsion phase was decanted and the solid residue of the microgels particles was washed twice with acetone and twice with an ethanol-water solution in the ratio of 1:1 by volume. After the washing, the microgels were rinsed with ethanol to remove all of the unreacted AG molecules, MBA and the surfactant molecules. Finally, the modified Arabic gun microgels (AGMAA) were store in the oven at 45 °C for further characterization and analysis.

Fourier Transform Infrared (FTIR) Spectroscopy Analysis

Starting materials including Arabic gum, methacrylic acid, polyethylene glycol dimethyl acrylate, polyethylene imine, N, N'-methylene bis-acrylamide, quercetin, doxorubicin, and the modified hydrogels and microgels were analyzed using FTIR-ATR (Perkin Elmer Spectrum 400) at 25 °C. The analysis was carried out in the wavenumber range of 4000 cm^{-1}– 500 cm^{-1} at a resolution of 2 cm^{-1}, the background was analyzed to serve as a control. Hydrogels and microgels were dried in an oven and powdered before the analysis.

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Swelling Properties

The study of swelling property of the hydrogel was performed at room (25 °C) and physiological temperature (37 °C) in media including deionized water, buffer solutions at pH 7.4 and pH 1.2, ionic solutions of NaCl, $CaCl_2$, and $AlCl_3$ of concentration 0.05 M to 0.25 M. The swelling ratio was determined by a known weighted amount of microgel and kept in each of the media separately. The microgel was removed from the medium at a specific time interval and the surface aqueous was wiped off. The weight of the gel was measured and returned to the medium. The process was repeated until a constant weight was reached. The swelling ratio at different time interval was calculated using equation 3.5.

$$SR = \frac{Wt-Wo}{Wo} \quad (1)$$

Where W_t is the weight at time t and W_0 is the initial weight of the hydrogels. This study was repeated thrice, mean value, standard deviation and error were calculated.

Differential Scanning Calorimetry (DSC) Analysis

Differential Scanning Calorimetry (Perkin Elmer) was used to determine the thermal properties of the microgels. About 7 - 10 mg of the dried powder microgel sample was weighed in an aluminium pan and an empty pan was used as a reference. The sample was scanned at a scanning rate of 10 °C min^{-1} under nitrogen atmosphere within the temperature range of 20-350 °C.

X-ray Diffraction (XRD) Analysis

This technique is mainly used to characterized materials by identifying the diffraction pattern. X-ray diffraction uses the dual nature of the X-ray particles/wave to obtain information regarding the crystallinity of the materials but not the composition. The variation in X-ray diffractogram arises due to the differences in the molecular arrangement between amorphous and crystalline powders. When X-ray light irradiates on lattice planes of crystalline materials, it scatters only in certain directions and results in high-intensity narrow peaks. The powdered microgels are not single crystals but composed of many crystallite units in the sample. The X-ray beam passes through the powdered sample and records the maximum interatomic planes. In this work, X-ray diffraction was used to analyze the crystallinity and amorphous nature of the microgel. The sample was analyzed using the Empyrean X-ray diffractometer. The samples were exposed to Cu Kα radiation with an accelerating voltage of 40 kV and a current of 40 mA at a scan rate of 1° min^{-1}. The diffraction angle was varied from 5° to 50° at 2θ, the analysis was performed at a temperature of 25 °C.

Field Emission Scanning Electron Microscopy (FESEM)

Field emission scanning electron microscopy is a non-destructive technique used to study the surface morphology, size and composition of materials. Images are capture as high energy electron scan over the sample and interact with atoms to generate signals which gives information about the surface morphology, size and compositional of the sample.

The surface morphology of the hydrogels and microgels samples were studied using Quant FEG 450 at a beam voltage of 5 kV. The samples were swelled in water for 24 hours before freeze-dried in liquid nitrogen for 48 hours. Dried samples were then coated with gold to avoid charging effect. At different magnification, the images of the surface were captured at the appropriate focus.

Degradation study of Microgels

The microgel materials were swollen in an aqueous buffer solution of pH = 7.4 phosphate buffer solution (PBS) and pH = 1.2 HCl acidic solution at 37 °C. Arabic gum polyethylene imine microgels were studied for their degradability at the different pH. The microgels were dried to constant weight, each of the formulations was weight before placing into 20ml of aqueous buffer solutions at 37°C and allowed to swell and degrade for six weeks (42 days). Readings were taken for the weight every 24 hours and the fresh buffers solutions previously equilibrated at 37 °C was replaced every 24 hours for the six weeks.

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The mass loss was monitored as the percentage of weight loss or percentage degradation of the microgels as shown in the equation (3.11).

$$\% \text{ Degradation} = \frac{M_t}{M_{max}} \times 100 \quad (2)$$

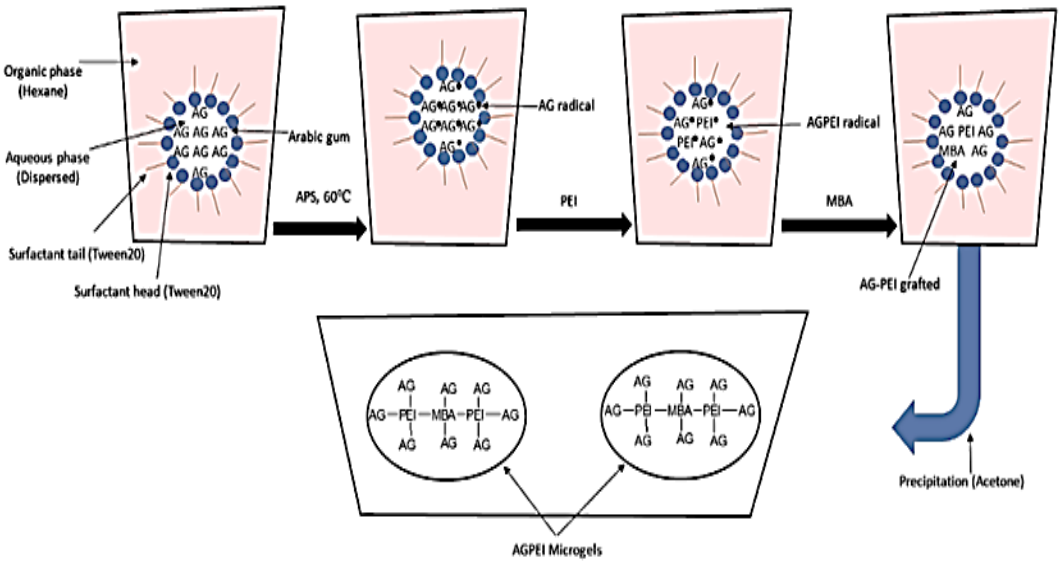
Where M_t is the weight at time t (swollen or dried) and M_{max} is the maximum swollen weight or the initial weight of the dried sample which varied from one microgel to other.

RESULTS AND DISCUSSIONS

Synthesis of Arabic gum-grafted-polyethylene Imine microgels

Arabic gum-grafted-polyethylene imine (PEI) microgels were synthesized by cross-linking the AG molecules with PEI polymer in micelle of the emulsion system, the surfactant creates a micelle of micro-size. The molecules of the gum and polyethylene imine reside in the inner parts of the micelles and addition of APS generates free-radical at 60 °C temperature by reacting with the carboxylic group of the gum and the primary or secondary amine group of the polymer to creates active radical sites on the AG and polymer molecules. The mixture is continuously stirred for grafting and polymerization of the radical active chains within the micelles of the emulsion which limit the size of the microgels formulated (Sahiner *et al.*, 2017; Sahiner *et al.*, 2017). Addition of cross-linking agent (MBA) into the emulsion system at constant stirring, terminate the chain propagation and finally cross-linked the macromolecule containing both the gum and polymer molecules (Ibrahim *et al.*, 2022). The microgel formed within the micelles contains the AG grafted with the PEI and cross-linked by the cross-linking agent. AGPEI microgel proposed scheme and digital image are shown in Figure 4.

(a)



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(b)

Figure 1.:
(a) Scheme of Arabic gum-grafted-polyethylene imine microgels formulation
(b) Digital image of AGPEI microgel

FTIR Analysis of Arabic gum grafted polyethylene Imine Microgels

Figure 2 shows the characteristics peaks of the FTIR spectra of (a) Arabic gum, (b) polyethylene imine. The FTIR spectrum of polyethylene imine grafted Arabic gum microgels is shown in Figure (2c) which indicates NH/OH stretching vibration at 3293 cm^{-1}, two stretching vibrations of C-H at 2921 cm^{-1} and 2850 cm^{-1} for symmetric and asymmetric respectively, N-H vibration at 1620 cm^{-1} and 1434 cm^{-1} for primary and secondary imine group, also the peak 1434 cm^{-1} corresponds to -CH_2 moieties (Sahiner et al., 2017).

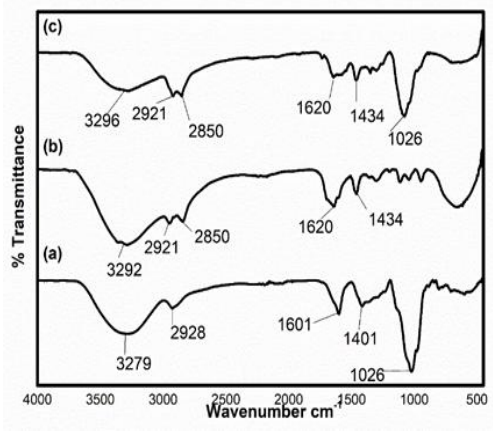

Figure 2: Spectrum of (a) Arabic gum, (b) Polyethylene imine and (c) AGPEI microgels

Swelling Property

Microgel swelling were studied in different media such as deionized water, buffer solutions (pH= 1.2 & 7.4) and salt/ ionic solutions at 25 °C and body physiological temperature (37 °C) to enable assessing the response of the microgels in different environmental conditions. The presence of primary, secondary, and tertiary amine in the polymer leads to the formation of a highly swellable microgel, the rate of swelling depends majorly on the polymer concentration. The microgel formulations are highly swellable in acidic buffer pH 1.2, follow by deionized water and the least swelling ratio was observed at pH 7.4 as shown in figure 3(a).

Microgels are environmentally sensitive materials; the pH of the media determines the swelling possibilities. The swelling of microgels was studied in distinctive buffer solution media of different pH 1.2 and 7.4, respectively. The interaction of the hydrogels functional groups in the media determines the rate of swelling, Nitrogen from the amine functional group of the polymer form hydrogen-bond with the acidic proton in the buffer solution pH 1.2. The hydrogen-bond between the amine and proton leads to polymer chain expansion which allows the absorption of water molecules and increases the rate of swelling ratio. Figure 3(a), the highest swelling ratio of the microgels was observed at pH 1.2 while the lowest swelling ratio is observed at pH 7.4 among the different media. Arabic gum grafted polyethyleneimine microgels shows higher swelling in an acidic buffer solution of pH 1.2 compare to a neutral or basic pH of 7.4.

Salts or ions solutions in aqueous medium produce cations and anions which are usually attached to the available oppositely charge molecules in media. Microgels formulated through emulsion polymerization proceed through proton abstraction which results in creating charged molecules and repulsion between this charge molecules lead to high swelling or absorption of water molecules. The presence of cations from the salt/ ionic solution reduces the repulsion between the negative charge polymer molecules which consequently lead to a reduction in swelling through shielding by the cations on the negative

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charge molecules. Depending on the ions concentration and strength Aluminium chloride ($AlCl_3$) shields more than calcium chloride ($CaCl_2$) which also shields more than sodium chloride (NaCl) as seen in Figure 3(b) & c respectively. Highly charged ions prevent swelling through shielding more than ions with less charge.

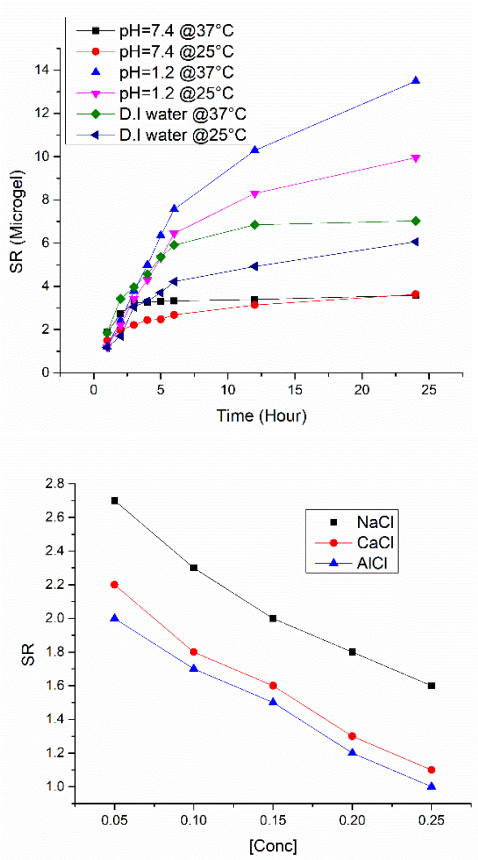

Figure 3: Swelling study of Arabic gum grafted polyethylene imine microgels in (a) different media pH (b) ionic solution

Microgels XRD Analysis

Figure 4. Illustrates the XRD diffractogram pattern of the formulated microgels. Figure 4 indicated a trend with a broad peak which was found only in modified microgels using polyethylene imine at 2θ = 15°-25°. This is due to the pattern of molecular rearrangement in the emulsion system and subsequent formation of the microgels. The distinctive sharp peak of the pure Arabic gum microgel as shown in Figure 3 indicates the absence of modifying agents and regularity in the newly formed a semi-crystalline structure with a characteristic sharp peak at 2θ = 20°.

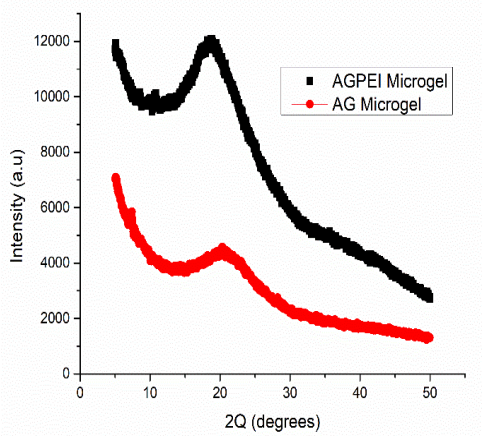

Figure 4: XRD Spectrum of (a) Arabic gum microgel (b) Arabic gum grafted polyethylene imine microgel.

DSC Microgel of Arabic gum cross-linked polyethylene imine

The DSC thermogram of the microgel for Arabic gum grafted polyethylene imine formulated is shown in Figure 5. The thermogram shows two peaks, endothermic peak at 199 °C and exothermic peak at 265 °C during the heating. The endothermic peak represents the melting temperature while the exothermic peak indicates decomposition temperature. The enthalpy changed for the melting is 174.5 J/g while the energy released during the process of decomposition is -223.5 J/g.

Arabic gum grafted polyethylene imine microgel formulation shows the highest temperature for melting at 199 °C with an enthalpy of 174.5 J/g. Microgels formulations consisting of long-chain polymers grafted onto the Arabic gum displayed higher melting temperature as shown by polyethylene imine formulations. Arabic gum molecules decomposition temperature supersedes that of all the formulated microgels as shown in Table 1.

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Table 1: Microgels formulations DSC thermogram temperature and enthalpies

Microgel formulation	Endothermic T_m (°C)	ΔH (J/g)	Exothermic T_p (°C)	ΔH (J/g)
AG	181	152.8	277	-25.4
AGPEI	199	174.5	265.5	-223.5

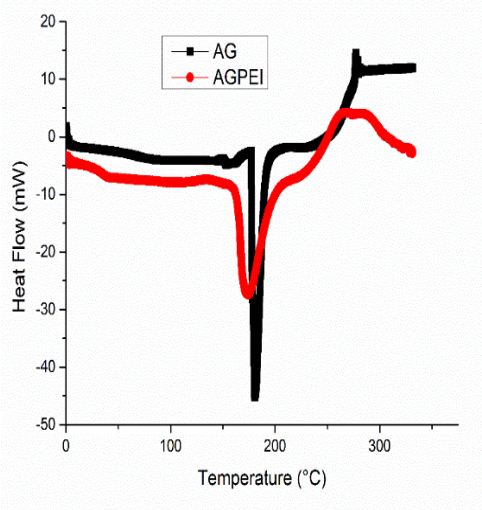

Figure 5: DSC Thermogram of (a) Arabic gum microgel, (b) Arabic gum grafted polyethylene imine microgel.

Morphology (Field Emission Scanning Electron Microscope)

The surface morphology of any material intended for drug delivery application is important. This is because the surface arrangement and geometry will affect the percentage swelling and the release behaviour of the microgel materials (Chen *et al.*, 2009; Kumar *et al.*, 2019). The surface morphology of freeze-dried microgel of Arabic gum grafted Polyethylene imine (AGPEI microgel) are in shown Figure 6. The micrograph reveals a nearly spherical shape for the microgels structure which appears to be connected between one particles to the other in a regular manner as shown in Figure 6. the uniformly distributed spherical shapes structure of the microgels are distributed all over the network surfaces.

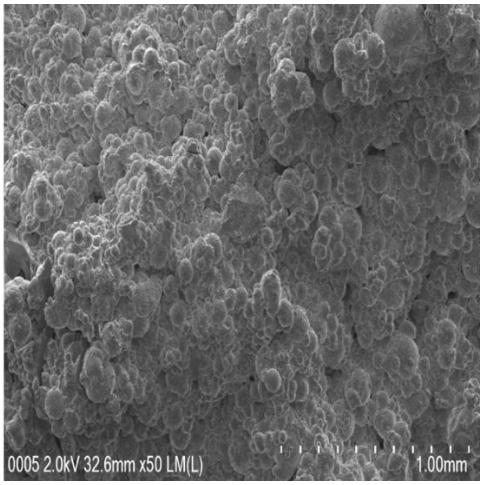

Figure 6: Micrograph of AGPEI microgel formulation

Degradation Studies

The microgels degradation studies were conducted and the result is shown in Table 2. At different mediums of pH 1.2 and 7.4 respectively. Generally, microgels formulations swell in the different mediums, but the rate of degradation depends solely on the pH of the media. At the buffer medium of neutral pH 7.4 and temperature of 37 °C, the microgel formulations swelled to the maximum capacity in 24 hours. The AGPEI microgels start degradation after 10 days in the buffer solution pH 7.4, on the sixth week of study (36 days) 46 % of AGPEI microgels were successfully degraded at pH 7.4 respectively (McBath *et al.*, 2010). The carboxylic group of the Arabic gum polymer in the microgel formulations is the main starting point of degradation at buffer solution pH 7.4.

The rate of microgel formulations degradation in a buffer solution of acidic pH 1.2 is distinctively different from a phosphate buffer solution of pH 7.4. AGPEI microgel formulations displayed a high percentage degradation in 6 weeks (36 days), 90.2 % of AGPEI were

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degraded, at the acidic buffer solution pH 1.2. The high degradation of AGPEI microgels in the acidic buffer solution pH 1.2, can be related to the expansion of the polymer network through the formation of a hydrogen bond between the atom of oxygen in PEG and nitrogen in PEI molecules of the microgel formulations respectively (Barszczewska-Rybarek, 2019; Selli *et al.*, 2019).

Table 2: Microgels degradation at buffer solutions pH = 1.2 and pH = 7.4 mediums

Microgels	Percentage degraded, %	
	pH 1.2	pH 7.4
AGPEI	90.2	46

CONCLUSION

Arabic gum as biopolymer under the class of carbohydrate like other polysaccharides has an uncontrolled rate of hydration and low mechanical strength which necessitate the modification for the formulation of microgels which are currently used for different industrial applications. In this work, Arabic gum was modified using synthetic polymer (polyethylene imine) to form microgels that are degradable and stimuli responsive. The formulated microgels were characterized using Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD) and differential scanning calorimetry (DSC) to proof the successful formulation of the microgels.

The microgels were formulated via emulsion polymerization using ammonium persulfate as initiator and N, N-Methylene bisacrylamide as the cross-linking agent. The concentration of the surfactant in the emulsion system plays an important role in determining the size of the microgel-particles. The microgels were found to be stimuli responsive by swelling in different mediums and we can therefore recommend the microgel for industrial application such removal dye and drug delivery in medicine and pharmaceutical.

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