

# Development and Evaluation of Alginate-gum Blend Mucoadhesive Microspheres for Controlled Release of Metformin Hydrochloride

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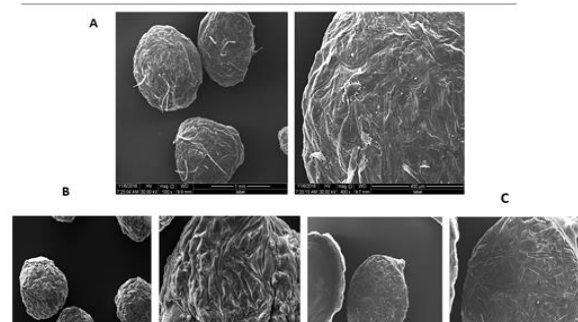
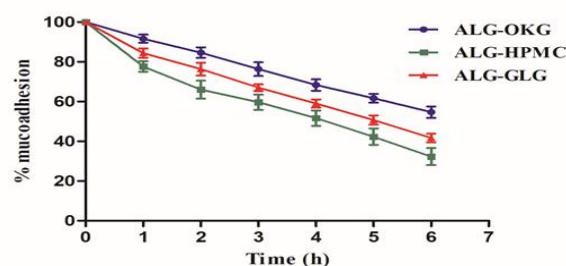
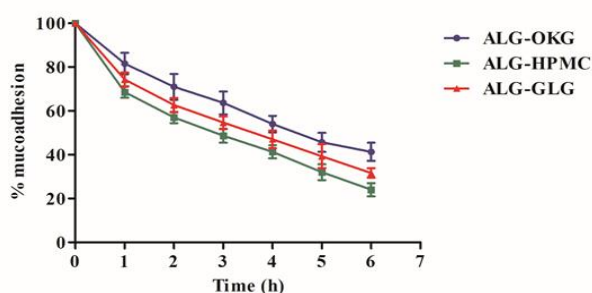
Received: January 31, 2021; revised: March 5, 2021; accepted: March 21, 2021

## Abstract

Microencapsulation is a tremendous technique that permits a compound to be included inside a microstructured system for several objectives including protection and sustained drug delivery. In our study, we prepared mucoadhesive microspheres of Metformin hydrochloride (MET) by ionotropic gelation approach using sodium alginate and calcium chloride as a cross-linker. Three natural polysaccharide copolymers e.g. Okra gum (OKG), Gellan gum (GLG), and Hydroxypropyl methylcellulose (HPMC) were added to alginate in a ratio of 1:1 to investigate their effect on mucoadhesion feature, drug encapsulation efficacy (EE %), and in-vitro drug release. Physical characterizations such as FTIR, XRD, DSC, particle size, and SEM were performed on the prepared microspheres. Ex-Vivo mucoadhesive property of the three tested microspheres was investigated using goat intestinal tissue. MET-loaded OKG-alginate microspheres (ALG-OKG) displayed a higher EE% ( $90 \pm 3.28\%$ ), more sustained release profile over 10 h and better adhesion to goat intestinal mucosa compared with the other microspheres using ALG-GLG or ALG-HPMC blend. Thus, the addition of isolated OKG to sodium alginate was manifested as a prospective controlled drug release polymer-blend in the formulation of sustained-release MET-ionically gelled microspheres for oral administration with expected enhanced bioavailability and better patient compliance due to a decreased dosage interval.

## Key words

Metformin hydrochloride, mucoadhesive microspheres, alginate, okra gum, Gellan gum, HPMC



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## Introduction

Diabetes is one of the main reasons for mortality in the world. Several antidiabetics are already utilized in practice, of which metformin hydrochloride (MET) is a vastly acceptable candidate. Unlike other antidiabetic drugs, MET does not motivate hypoglycemia at any rational dose, and consequently, the term "anti-hyperglycemic" is more accurate to be used for MET rather than a hypoglycemic drug. Regardless of its affirmative therapeutic outcome and rareness of critical defects, chronic treatment with MET encounters some drawbacks such as its large dosage (1000-2000 mg/day), reduced bioavailability (60%), and probable occurrence of gastrointestinal undesirable effects. Subsequently, there are persistent attempts to enhance the drug delivery of MET to obtain an optimal remedy. The essential focus of these attempts was to sustain the drug release involving the long-practiced gastro-retentive formulations. However, the main problem was the decrease in drug absorption in presence of food. Thus, the logical thought directs to controlled release/sustained release CR/SR delivery systems of MET (1). However, CR delivery form resulted in a reduction of bioavailability of the drug, it could be attributed to the fast passage of the CR single dose from the absorption site prior to the drug release and most of the drug released at the colonic region in which MET is poorly absorbed (2,3). Therefore, the appropriate form for MET is supposed to be gastro-retentive formulations (1,4), from which the drug releases gradually in the stomach for slow absorption in the intestine. An improvement of the bioavailability of the drug will be expected due to the delayed but complete release of the drug and hence its entire usage which may lead to a smaller dose with diminished GI side effects. Mucoadhesive microspheres are designed to adhere to mucosal tissues, retain the drug for a long time and permit its release at a sustained rate while decreasing dose dumping (5).

Many natural biodegradable polymers such as sodium alginate (ALG), okra gum (OKG), Gellan gum (GLG), and hydroxypropyl methylcellulose (HPMC) are extensively used for the delivery of several bioadhesive formulations. In pharmaceutical research, the gel-forming features of the previously mentioned polymers in presence of multivalent cations have been considered for sustaining the release of drugs (6–11).

Alginates (ALG) are vastly utilized in the nutrition and pharmaceutical industries because of their viscosity-enhancing, non-toxic, and gel-forming features. Alginates are a category of anionic linear polysaccharides created from brown algae and certain bacteria. Also, it has been thoroughly used for oral immunization by targeting the Peyer's patches in the small intestine (12). Alginic acid is a copolymer with homopolymeric blocks of (1-4)-linked  $\beta$ -D-mannuronic (M) and its C-5 epimer  $\alpha$ -L-guluronic (G) residues, respectively, covalently attached and organized in various sequences viz, homopolymeric residues (M or G) or heteropolymeric residues (MG) (13).

Calcium alginate beads are previously experienced for their limited drug entrapment efficiency, rapid deterioration, and quick burst intestinal drug release (14,15). These drawbacks encourage scientists to make modifications to the alginate particles in order to enhance these drawbacks and control the release. Recently, many researchers modified the alginate beads by applying various polymer blends of sodium alginate and other polymers (15–22).

Okra gum (OKG), an inert, non-irritant biopolymer separated from *Hibiscus Esculentus*, is a polysaccharide consisting of D-galactose, L-rhamnose, and L-galacturonic acid (23,24). It forms a viscous solution when dissolved in water with a gluey semblance (25). OKG is previously examined as a beneficial excipient in the formulation of many delivery systems as a suspending agent, a granulating and binding agent (26–28). In addition, OKG is used as emulsifying (26,27). OKG has bioadhesive properties as well it has many considerations as antioxidant and antidiabetic agent (28). The highly viscous property of OKG leads to its utility as a drug-release sustaining polymer (29).

Gellan gum (GLG) is the newly available gelling agent used in the food and pharmaceutical industries. GLG is an anionic hetero-polysaccharide produced by certain bacteria (30). GLG is capable of forming gels with polycations at low concentrations; hence, its utility has broadened, and it is used in many formulations as a viscosifier and stabilizer (31).

Hydroxypropyl methylcellulose (HPMC) is a synthetic, nonionic polymer. It has been widely studied as a mucoadhesive polymer by many investigators on account of its elevated consistency at low concentrations and safely used with regard to toxicity (32).

Hence to attain the essential aim of preparing a therapeutically effective drug delivery system of MET for efficient management of Non-Insulin Dependent Diabetes Mellitus, this study was destined to fulfill the following goals: formulation of mucoadhesive microspheres through ionotropic gelation technique using a blend of bioadhesive polymers such as sodium alginate/okra gum blend, sodium alginate/Gellan gum blend, and sodium alginate/HPMC blend; characterization of the prepared microspheres by FTIR, DSC, XRD, and SEM; in-vitro release patterns of the drug via these formulations and an estimation of the adhesion property of the prepared microspheres.

## Materials and Methods

### Materials

MET was kindly presented by MUP (Medical Union Pharmaceuticals), Abu-Sultan, Ismailia, Egypt, OKG was separated from okra fruits as previously reported (25). Sodium alginate, HPMC, Gellan, and  $\text{CaCl}_2$  were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA), other chemicals and solvents were of commercial analytical grade.

### Preparation of OKG

OKG was obtained based on the previously reported procedures with some modifications (25). One kg of immature and soften Okra fruits was rinsed and cut as thin slices while removing the seeds from the fruits. The segmented bloc was impregnated in plenty of water for one night. The produced viscous mucilage was filtered out through a muslin cloth. The gum was deposited as a precipitate using acetone at acetone: gum ratio of 3: 1. Finally, the gum residue was desiccated at 40°C. The produced residue was ground, sieved through a mesh no. 120 and retained in a desiccator until used.

### Preparation of MET-loaded mucoadhesive microspheres:

Drug-loaded mucoadhesive microparticles were formulated using the ionotropic gelation technique which enclosed an interaction between sodium alginate and polyvalent cation such as calcium to form a hydrogel composite of calcium alginate.

MET (100 mg) was dissolved in 50 ml water using a magnetic stirrer at 100 rpm then add sodium alginate (ALG) to the drug solution followed by the mucoadhesive copolymer (OKG, GLG, HPMC) in a ratio of 1:1 alginate to the mucoadhesive polymer to prepare a concentration of 2% w/v polymer solution, keeping drug to polymer ratio 1:2. Using a homogenizer at 1000 rpm for 10 min, the whole drug-polymers dispersion was homogenized until forming a homogeneous viscous polymer blend/drug dispersion. The prepared dispersion was then dropped through a 22G needle into 100 ml calcium chloride (8% w/v) solution as a cross-linker with continuous stirring. Ionic gelation will be occurred for polysaccharides to produce spherical gels because of the complexation between oppositely charged species. The formed gel spheres were kept in the cross-linking solution for 0.5h as a curing time to form rigid and consistent gel microparticles. By decantation, the prepared gel spheres were collected, rinsed repeatedly with purified water for removal of any calcium deposits then air-dried and stored in a desiccator until used.

### Drug Entrapment Efficiency (EE%)

50 mg of each formulation of MET-loaded microspheres were accurately weighed separately, ground, and transmitted in a 100-ml volumetric flask with purified water and retained under stirring for 24 h. for the equilibrium objective. The produced polymer residue after smash of microspheres was filtered using filter paper. Using the filtrate, we could determine the drug content spectrophotometrically at  $\lambda_{\max}$  of 232 nm using (6800 UV/Vis. Spectrophotometer, JENWAY, UK). The entrapped drug in the prepared microspheres was calculated as follows:

$$EE \% = \frac{\text{Actual amount of drug present}}{\text{Theoretical drug amount predicted}} \times 100$$

### Yield Percentage

The production yield percent was assessed from the produced weight after drying of each formulation and the total initial weight of the original components. The yield percent was determined by the following equation:

$$\% \text{ Yield} = \frac{\text{Practical mass (microspheres)}}{\text{Theoretical mass (polymers + Drug)}} \times 100$$

### Estimation of Particle size

Particle size analysis was estimated by optical microscope using stage micrometer slide.

### Scanning electron microscopy (SEM)

A scanning electron microscope was used to inspect the morphological features of the prepared microspheres, (INSPECT S50, FEI, USA).

### Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of the prepared microspheres (ALG-OKG microspheres, ALG-GLG microspheres, and ALG-HPMC microspheres) along with the pure drug and the utilized polymers were obtained and compared at a scanning speed 4000-200  $\text{cm}^{-1}$  using a (TENSOR 37, BRUKER, GERMANY) using potassium bromide disc method.

### X-ray diffraction (XRD) analyses

The crystallinity or amorphous nature of the samples chosen for FTIR was investigated. The XRD patterns were obtained utilizing an (XMD-300, UNISANTIS, GERMANY) equipped with Cu K $\alpha$  radiation tube operated at 45 kV, 0.8 mA and the scan range was 5–65° of the diffraction angle 2 $\theta$ , in steps of 0.04 and scanning speed of 0.4 degrees/second. To avoid external factors, equal amounts of samples were used for the study.

### Differential scanning calorimetry (DSC)

Thermal behavior of plain drug powder, the utilized polymers, and the formulated microspheres was carried out using (DSC-60, SHIMADZU, JAPAN). The samples were sealed in aluminum pans and heated under a flow of nitrogen (30 ml/min) using a scanning rate of 10 °C/min from 25 - 250°C using an empty pan as a control.

### In-vitro Drug Release Study

In-vitro drug release profiles were carried out using 100 mg of pure drug or its equivalent weight of the prepared microspheres using USP type II dissolution test apparatus (COPLEY, UK). The dissolution conditions were adjusted in 0.1 N HCl for 2 h then in phosphate buffer pH 6.8 for a further 8 h at 37°C  $\pm$  0.5 °C and at 50 rpm. At particular time intervals, 2 ml of the dissolution media was withdrawn, filtered and suitably diluted. Then it was assayed spectrophotometrically for the drug content (6800 UV/Vis. Spectrophotometer, JENWAY, UK) at  $\lambda_{\max}$  232 nm against an appropriate reference. For the sink condition objective, an equal volume of fresh dissolution medium was substituted following each sampling.

### Modeling of drug release profiles

Various mathematical patterns were kinetically fitted for the In Vitro release results of the prepared MET-microspheres such as: Zero-order, First-order, Higuchi's model, Hixson- Crowell models, and Korsmeyer-Peppas. The regression analysis of all plots is obtained to calculate the regression coefficient (R<sup>2</sup>), K (kinetic constant) values, diffusional release exponent (n) and half time ( $t_{1/2}$ ) (33,34).

### Ex-Vivo evaluation of mucoadhesion feature:

The mucoadhesive feature of the prepared MET microspheres was evaluated by the previously reported technique known as the Ex-Vivo wash-off approach (35). An intestinal mucosa from freshly slaughtered goat were cut into pieces (2 x 2 cm) and fixed on glass slides (7.5 x 2.5 cm) using glue. Then about 50 microspheres from each polymer form were spread and attached on the wet tissue pieces separately and instantly attached to USP II tablet disintegration test apparatus (COPLEY, UK) using simulated intestinal fluid at pH 6.8 at 37 °C as disintegration media. Upon operating the tester, the slide undergoes up and down slow movements in the test fluids, 900 mL. At specified time intervals, up to 6 h, the apparatus was stopped and the retained number of adhering microspheres on each slide were counted. The experiment was repeated thrice on each type of the prepared microspheres to calculate the average number.

$$\% \text{ of mucoadhesive} = \frac{\text{No. of remaining microparticles on mucosa}}{\text{Total No. of applied microparticles}} \times 100$$

### Statistical analysis:

Statistical analysis was performed using Graphpad Prism 5 software. All measured data were expressed as mean  $\pm$  standard deviation (S.D). One-way ANOVA test was used to compare the three prepared formulations. ( $P < 0.05$  was considered significant).

### Results and Discussion

A rapid, easy, and cost-effective method was selected to prepare the mucoadhesive microspheres using polymer blends of sodium alginate with OKG, GLG, or HPMC copolymers and calcium chloride as a cross-linking agent to formulate mucoadhesive microspheres by ionotropic gelation technique.

Mucoadhesive dosage forms are delivery systems that depend on the advantages of bioadhesion of some polymers which adhere to the mucous membrane on hydration, and this bioadhesion phenomenon may be mainly due to intermolecular interaction amongst like molecules. And this property can be applied for delivering a certain medication to a particular region of the body for expanded timeframes according to its specific site of absorption.

### Determination of particle size and drug entrapment efficiency (EE%)

The size of the formulated microspheres was ranged between  $0.682 \pm 0.11$  and  $0.831 \pm 0.15$  mm (Table 1). The large particle size of all formulations is expected due to the high polymer concentration of the polymer blend of alginate with the used copolymer. An observed increase in the viscosity of the medium, as well as the high availability of calcium binding sites in the polymeric chains, are attributed to the high concentration of the polymer mixture. Consequently, the degree of crosslinking also increases (36) and bigger droplets were produced.

The entrapment efficiency of MET in the prepared ALG-OKG, ALG-HPMC, and ALG-GLG microspheres was found to be  $92.5 \pm 3.28\%$ ,  $86.3 \pm 2.12\%$ , and  $42.1 \pm 3.25\%$ , respectively (Table 1). The utilized concentration of  $Ca^{2+}$  ion together with the high viscosity of the polymer medium induces high cross-linking of alginate and produces insoluble dense matrices, which leads to diminished leakage of the drug to the cross-linking solution and hence, high drug entrapment (37) in the ALG-OKG and ALG-HPMC microspheres. Larger pores and lower viscosity due to insufficient cross-linking might share in drug leaching throughout these pores in case of ALG-GLG microspheres. This may lead to lower drug entrapment in the ALG-GLG matrices (34). The same results were reported in the preceding studies on the preparation of ionically-gelled microspheres formulated utilizing ALG-other copolymers mixtures (17,18,22,38–40).

**Table 1: Physical characterization of the prepared microspheres**

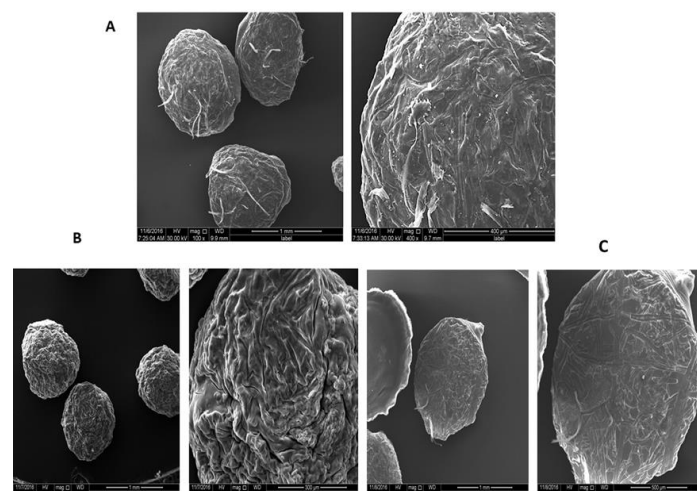
Factor	AIG-OKG microspheres	AIG-HPMC microspheres	AIG-GLG microspheres
Entrapment Efficiency %	$92.5 \pm 3.28\%$	$86.3 \pm 2.12\%$	$42.1 \pm 3.25$
% Yield	97.6%	95.3%	98.7%
Size of microsphere (mm)	$0.831 \pm 0.15$	$0.711 \pm 0.23$	$0.682 \pm 0.11$

### Surface morphology of the prepared microspheres

SEM analysis of the samples detected that all the formulated microspheres were spherical in shape. Figure 1 presents the morphology of MET-loaded ALG-OKG, ALG-GLG, and ALG-HPMC microspheres with their magnification.

Upon examination of both ALG-OKG and ALG-GLG microspheres, the outer surfaces of these microspheres appeared heterogeneous, irregular, rough, and porous which can be attributed to the water loss during the solvent evaporation step. However, smoother surfaces were observed on the ALG-HPMC microspheres. The morphological features of the formulated (ALG-OKG and ALG-GLG) microspheres showed very harsh texture with distinctive wrinkles, which might be attributed to a partial shrinkage of the gellified polymer matrix upon drying (20). During the drying process, the formed crust on the surface of the droplets inhibits the vaporization of the solvent leading to the growth of the vapor pressure. Consequently, tiny pores and channels are created (41). Surface clefts could be due to the subsequent collapse of the droplets after solid particles are assembled. The observed surface morphology may have an important relation to the bioadhesion feature. It has been reported that microspheres with a coarser and more porous texture may display an intensified mucoadhesive property compared with those with a smoother surface (42). Therefore, the observed rough, coarse texture in the ALG-OKG and ALG-GLG microspheres may have led to their higher bioadhesion as compared with the ALG-HPMC microspheres. It can be seen that there is a formation of deeper cracks/channels on the surface of the ALG-GLG microspheres which may also explain the lower drug entrapment in these microspheres.

Little debris of polymers and crystals of the drug were observed on the surface of the microspheres. The appearance of polymeric debris on the surface of the microspheres could be attributed to the concurrency between gel bead preparation and the construction of the polymeric blend matrix; whilst the appearance of crystals of plain MET on the surface of the microspheres might be framed due to their emigration alongside aqueous droplets to the surface throughout the desiccation stage (43,44).

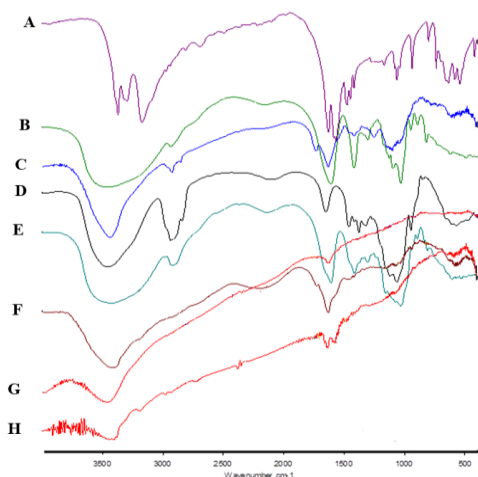


**Figure 1: Scanning Electron Micrographs of the formulated MET-loaded microspheres (A) ALG-OKG microspheres, (B) ALG-GLG microspheres, (C) ALG-HPMC microspheres**



## Characterizations of the prepared microspheres FTIR analyses

FTIR spectral analyses of pure MET, alginate, OKG, HPMC, GLG, and the formulated microspheres (ALG-OKG, ALG-HPMC, and ALG-GLG) are displayed in **Figure 2**. The FTIR spectrum of the pure drug displayed two characteristic absorption bands at  $3371.82\text{ cm}^{-1}$  and  $3297.05\text{ cm}^{-1}$  which are corresponding to the N-H primary stretching vibration and a characteristic band at  $3175.19\text{ cm}^{-1}$  which is attributed to the N-H secondary stretching, in addition to two characteristic bands at  $1628.39\text{ cm}^{-1}$  which referred to C-N stretching, and a band at  $1566.84\text{ cm}^{-1}$  appears due to N-H bending vibrations of the primary amine group.



**Figure 2:** FTIR spectra of (A) pure MET, (B) ALG, (C) OKG, (D) HPMC, (E) GLG, (F) ALG-OKG loaded microspheres, (G) ALG-HPMC loaded microspheres, and (H) ALG-GLG loaded microspheres.

The peculiar bands of sodium alginate in the FTIR spectrum were displayed at  $1417\text{ cm}^{-1}$  and  $1609\text{ cm}^{-1}$ , which is representative for symmetric and asymmetric C=O stretching vibrations of  $\text{-COO}^-$  anions, respectively. Furthermore, a broad band exhibited at  $3448\text{ cm}^{-1}$  which is referred to the -OH stretching vibrations.

The spectrum of the pure OKG exhibited a weak band at  $1417\text{ cm}^{-1}$  which is corresponding to C-H bending, another weak band at  $1630\text{ cm}^{-1}$  which represented to C=O stretching, a weak band at  $2923\text{ cm}^{-1}$  due to C-H stretching band and a wide broad band at  $3442\text{ cm}^{-1}$  which represented to -OH stretching vibrations.

The FTIR spectrum of GLG displayed a principal peak around  $3555\text{ cm}^{-1}$  which represented to stretching of OH, peak at  $3500\text{--}2923\text{ cm}^{-1}$  referred to C-H stretching,  $1608\text{ cm}^{-1}$  referred to C-O stretching,  $1414\text{ cm}^{-1}$  for methyl C-H bonding, and  $892\text{ cm}^{-1}$  for C-O stretching of alkyl ether.

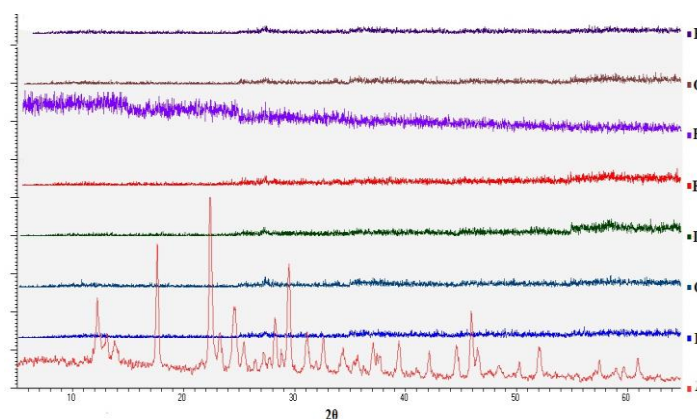
The FTIR spectrum of HPMC showed a broad band at  $3467\text{ cm}^{-1}$  corresponding to OH stretching vibrations; another band at  $2935\text{ cm}^{-1}$  refers to the stretching vibrations of methyl and Hydroxypropyl groups. The bending vibration band at  $1650\text{ cm}^{-1}$  is representative of H-OH groups and the stretching vibrations of C=O groups. An obvious broadened band is displayed in the range  $1100\text{--}1000\text{ cm}^{-1}$  is corresponding to the C-C and C-O vibrations of cellulose (45).

In the FTIR spectrum of the formulated microspheres, several principal bands of MET, ALG, OKG, HPMC, and GLG were manifested with no significant shifting or deviation. Thus, the obtained FTIR results may propose that there is no significant

interference or interaction between MET and the polymers utilized in the microsphere formulation.

## XRD analyses

The diffractogram patterns of the plain drug (MET), the prepared microspheres, and the utilized polymers (alginate, OKG, HPMC, and GLG) showed in **Figure 3**. XRD of MET shows sharp intense diffraction peaks at  $2\theta$  angles of;  $17.28^\circ$ ;  $22.18^\circ$ ;  $23.04^\circ$ ;  $29.08^\circ$ ;  $30.83^\circ$ ;  $33.90^\circ$ ;  $36.83^\circ$  and  $39.08^\circ$ , which indicate the crystalline state of the pure drug. The diffraction patterns for alginate, GLG, HPMC, and OKG, all showed the amorphous nature of the polymer used. However, the intensity of significant peaks of MET was decreased in the XRD patterns of the formulated microspheres (ALG-OKG, ALG-HPMC, and ALG-GLG microspheres) which indicates a remarkable decrease in the crystallinity of the drug or the conversion of the crystalline state of MET to amorphous state within the formulated microspheres. The crystallinity change of MET within the formulated microspheres could be attributed to progressive amorphization and/or dissolution of the drug inside the polymer blend matrix during preparation.



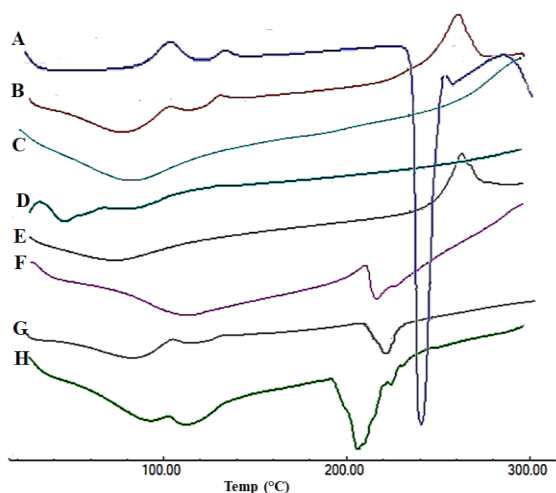
**Figure 3:** XRD patterns of (A) pure MET, (B) ALG, (C) OKG, (D) HPMC, (E) GLG, (F) ALG-OKG loaded microspheres, (G) ALG-HPMC loaded microspheres, and (H) ALG-GLG loaded microspheres.

## Differential Scanning Calorimetry (DSC)

Thermal behaviors of the formulated microspheres and their pure components (MET, ALG, OKG, HPMC, and GLG) are displayed in **Figure 4**. The thermogram of pure MET showed a characteristic sharp endothermic peak appeared at  $242^\circ\text{C}$ , which is attributed to its melting point, indicating its pure crystalline form. The DSC thermograms for the pure polymers indicated typical amorphous materials, they show broad large dehydration bands in the  $50\text{--}100^\circ\text{C}$  temperature range. The DSC thermograms of the prepared microspheres were practically the sum of the pure components, but the characteristic endothermic peak of the drug showed a slight shift to  $220$ ,  $220$ , and  $210^\circ\text{C}$  in the ALG-OKG, ALG-HPMC, and ALG-GLG microspheres respectively. This may be attributed to the molecular dispersion of the drug in the polymer matrices, in addition to the change in the crystallinity nature of the drug during formulation which is approved in the XRD analyses (46).

These results match the results obtained from FTIR studies, which indicate that there is no chemical interaction between the drug and the utilized polymers.

**Figure 4: DSC thermograms of (A) pure MET, (B) ALG, (C) OKG, (D) HPMC, (E) GLG, (F) ALG-OKG loaded microspheres, (G) ALG-HPMC loaded microspheres, and (H) ALG-GLG loaded microspheres.**



### In Vitro release

The results of the in-vitro release of the drug are shown in **Figure 5**. It can be observed that, for all the prepared microspheres, the amount of drug released from microspheres in the simulated gastric medium, low pH 1.2 were significantly less than the amount released in the simulated intestinal medium, high pH 6.8, and this may be mainly due to the shrinkage of calcium alginate in lower pH and poor solubility of alginate microspheres in the lower pH, which leads to decreased drug release from the microsphere matrices. While in an alkaline medium, the calcium alginate matrix shows a high swelling index and high solubility of calcium alginate, which permits the rapid release of the entrapped drug.

ALG-OKG microspheres were found to be more significantly effective in controlling the drug release over 10 h as there was a highly significant difference upon comparison with ALG-HPMC and ALG-GLG microspheres ( $P < 0.0001$ ) which may be attributed to the production of rough and hard okra coat when compared to the other mucoadhesive polymers (HPMC and GLG). Also, this effect of OKG can be due to its higher hydrophilic property leading to the formation of a highly viscous gel structure upon binding with water, which may be partially blocking the surface pores of the microspheres producing more sustained release of the drug (35). Among all the formulations, ALG-GLG microspheres showed a faster dissolution profile (**Figure 5**). The ALG-GLG microspheres are pH-sensitive as they are stable in low pH; however, they become unstable in higher pH because they rapidly swell in high pH than low pH (6). This feature leads to an early release of the associated drugs from several GLG microspheres in the intestinal pH. The addition of other biodegradable polymers with ionotropically-gelled Gellan microspheres has been investigated as a widespread way to decrease this limitation (47,48).

To get more information about the mechanism of drug release, the results of In Vitro drug release study were fitted into different mathematical kinetic models (**Table 2**). The mechanism of drug release from the formulated microspheres is fit to zero-order model which indicates that the drug release is independent on the concentration.

The Korsmeyer–Peppas model indicates that the drug release from ALG-OKG microspheres follows a case II transport model ( $n = 1.01$ ) which involves polymer relaxation and chain disentanglement while the release from ALG-HPMC and ALG-GLG microspheres is non-Fickian release (anomalous transport) and  $n$  value is between 0.5 -1 which is controlled by a combination of diffusion and polymer relaxation (49,50).

**Table (2): Kinetic parameters of the In Vitro release of MET from the three formulated mucoadhesive microspheres (ALG-OKG, ALG-HPMC and ALG-GLG microspheres)**

	Zero order	First order	Higuchi	Hixson-crowell	Korsemyer-Peppas
<b>ALG-OKG microspheres</b>					
<b>R<sup>2</sup></b>	<b>0.99216</b>	-0.9394	0.954283	0.965258	0.983667
<b>k</b>	0.151978	-0.00341	4.454673	0.003898	<b>n</b>
<b>t(1/2)</b>	328.995	-203.04	125.9819	245.2923	1.01
<b>ALG-HPMC microspheres</b>					
<b>R<sup>2</sup></b>	<b>0.99525</b>	-0.83624	0.991508	0.931263	0.997015
<b>k</b>	0.192846	-0.01243	5.324675	0.007882	<b>n</b>
<b>t(1/2)</b>	259.2736	-55.7739	88.17668	121.3106	0.82
<b>ALG-GLG microspheres</b>					
<b>R<sup>2</sup></b>	<b>0.986096</b>	-0.88552	0.976895	0.968375	0.983094
<b>k</b>	0.209307	-0.01324	5.746846	0.008612	<b>n</b>
<b>t(1/2)</b>	238.8834	-52.3379	75.69739	111.0286	0.79

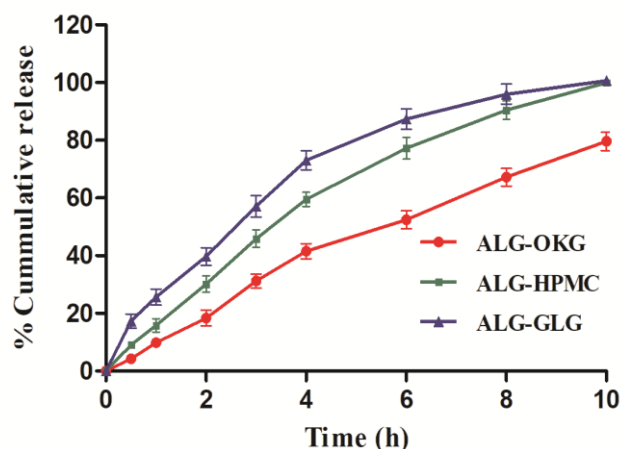
R<sup>2</sup>: Regression coefficient

k: kinetic constant

t(1/2): half time (min)

n: the diffusional release exponent

**Figure 5: In Vitro release pattern of the formulated MET-loaded microspheres in 0.1 N HCl (pH 1.2) for first 2 h and then in phosphate buffer (pH 6.8) for next 8h.**



### Ex-Vivo evaluation of mucoadhesion feature

MET is mainly absorbed from the small intestine. Therefore, optimizing oral MET therapy requires the development of a formula that can confine to a specific site in the upper intestine. Hence, mucoadhesive microspheres can meet this requirement and be a suitable formulation for MET (51,52). The mucoadhesion is a valuable feature as it may avoid rapid peristaltic elimination of the administered drug formulation and hence, permits prolonged contact with the absorption site (53). The results of Ex-Vivo wash-off studies are shown in **Figure 6**. These results indicated that all the formulated microspheres possess high mucoadhesive properties, where the viscosity of the mixture increased upon the blending of alginate with the other mucoadhesive polymers (OKG, HPMC, and GLG), forming a highly viscous gel which assists to increase the attachment of microspheres with mucosa. The mucoadhesive activities of the prepared microspheres can be a result of the presence of the -OH in both sodium alginate and the (OKG, HPMC, and GLG), which tend to form hydrogen bonds with the adjacent hydrated mucus membrane molecules (54). The adhesion of the three formula were compared and revealed that ALG/OKG microspheres have the highest percentage of mucoadhesion with a statistically significant difference between the three formula ( $P < 0.0001$ ).

It was found that ALG-HPMC microspheres show fewer mucoadhesion features as compared to ALG-OKG and ALG-GLG microspheres. This may be attributed to the observed rough, coarse texture of ALG-OKG and ALG-GLG microspheres as compared with the ALG-HPMC microspheres. Thus, the formulated microspheres adhere to the mucosa for a prolonged period where they release the drug in a sustained way before being disintegrated. The enhanced mucoadhesive property of MET loaded ALG-OKG microspheres could provide more advantages for sustaining the drug release as the main absorption site for MET is the proximal upper intestine. In addition, the highest mucoadhesion properties together with the highest EE% of OKG made it superior to HPMC and GLG.

### Conclusion

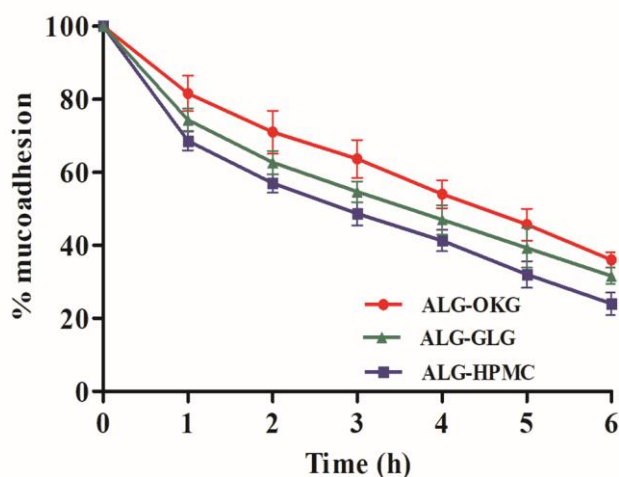
It has been reported that effective mucoadhesive systems could enhance bioavailability by safeguarding the drugs from physical and chemical deterioration, improving the absorption rates by decreasing the diffusion barriers, and increasing the interval of absorption by the prolongation of the residence time. Sustained release mucoadhesive microspheres were prosperously formulated using ionotropic gelation method by polymeric blends of sodium alginate with OKG, GLG, or HPMC as mucoadhesive polymer. The formulated microspheres were spherical in shape with a harsh surface in case of ALG-OKG and ALG-HPMC microspheres and smoother surface in case of ALG-GLG microspheres. The drug entrapment efficiency was found to be in the following rank: ALG-OKG > ALG-HPMC > ALG-GLG. The ALG-OKG microspheres were found to be more efficient in sustaining the drug release as compared to the ALG-HPMC and ALG-GLG microspheres. Among all the formulations, ALG-OKG microspheres displayed a better dissolution profile with about 88 % drug released controlled for 10 h. The kinetic data fit zero-order kinetics which referred that the mechanism of the drug release is independent on the concentration with an indication that the drug release involves polymer relaxation and chain disentanglement in case of ALG-OKG microspheres but includes drug diffusion and polymer relaxation in case of ALG-HPMC and ALG-GLG microspheres. The use of OKG was proved to be a potential sustained drug release matrix when blended with sodium alginate for the development of MET-loaded microspheres, which may enhance the oral bioavailability with reduced dosing interval and GI side effects (as less chance of dose dumping), with improved patient compliance.

### Declarations

### Conflict of interest:

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

**Figure 6: Mucoadhesion Ex-Vivo study of the formulated microspheres in simulated intestinal fluid pH 6.8 at 37°C**



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