Liraglutide Drug Loading with Double Layered Hydroxide- Synthesis and Characterization

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Abstract:

The liraglutide, antidiabetic drug, was loaded with layered double hydroxide nanoparticles for selective ions such as Fe^{+3}, Fe^{+2}, Ni^{+2} and Al^{+3}, the LDH were prepared through the titration method by adding 2M of NaOH to the mixture of trivalent, divalent ions and 0.5M HCl, the drug liraglutide was added to Prepared layered. The compounds were characterized by SEM, AFM, FT-IR, XRD and Zeta potential technique.

Key words: liraglutide, LDH, nanoparticales

تحضير وتشخيص دواء اليراكلوتايد المحمل في طبقات نانوية

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الخلاصة:

اليراكلوتايد هو علاج لداء السكري, تضمن العمل تحضير نواقل لايونات ثنائية وثلاثية التكافؤ لايونات الحديد والالمنيوم والنيكل ، حيث حضرت هذه النواقل من خلال عملية التسحيح بأضافة 2 مولر من هيدروكسيد الصوديوم الى خليط الحديد الثلاثي مع النيكل الثنائي والى خليط الحديد الثنائي مع الألمنيوم الثلاثي على التوالي بوجود 0.5 نورمالية من حامض الهيروكلوريك مع اضافة دواء اليراكلوتايد لتحميله على الطبقات المحضرة. جرى تشخيص المركبات المحضرة بعدة طرق طيفية مثل الاشعة تحت الحمراء وانكسارات حيود الاشعة السينية ومجهرالماسح الالكتروني وكذلك مجهر القوى الذرية للجزيئات كما تمت قياس مجهادية زيتا للجزيئات لمعرفة حجوم وحركية الدقائق للمركبات المحضرة.

الكلمات المفتاحية: ليراكلوتايد، طبقات الهيدروكسيد الثنائية، الجزيئات النانوية..

Introduction

Layered double hydroxides (LDHs), also known as anionic clays or hydrotalcite-like compounds, represented by the general formula: $[M^{II}_{1-x}M^{III}_{x}(OH)_2]^{x+}[(A^{n-})_{x/n} \cdot yH_2O]^{x-}$, where M^{II} and M^{III} are di-and tri-valent metal cations, respectively, are capable of occupying the octahedral interspaces of brucite-like sheet. A^n is anions between the layers can compensate the positive charges of the layer structures and these interlayer regions may contain H_2O molecules [1]. Owing to the rich intercalation chemistry of LDHs, these materials have extensive applications as catalysts, flame retardants, adsorbents, optical and electric functional materials and polymer stabilizers. In the recent time, particular attention has been focused on the LDH in the medical field and especially on LDH-based controlled release systems [2]. Although many biomolecules-LDH hybrid complexes have been reported, only a few examples have been studied as drug

delivery agent such as diclofenac sodium and ibuprofen [3,4].

Liraglutide is a fatty acid derivative of glucagon like peptide -1 (GLP-1) with chemical formula ($C_{172}H_{265}N_{43}O_{51}$) prepared by attaching a 16-carbon fatty acid molecule at Lys26 position and making an Arg34Lys substitution on GLP-1, and shares almost 97% sequence homology with GLP-1. These structural modification of liraglutide resist DPP-IV degradation, increase chain aggregation and enhance reversible non-covalent binding to other molecules, such as albumin. Liraglutide marketed as Victoza, is a 1.8 mg daily subcutaneous injection that was initially approved by the FDA in 2010 as an adjunct therapy to diet and exercise for management of type 2 diabetes.

In the present study, liraglutide selected as a model drug and intercalated into Fe–Al-LDH and Ni-Fe- LDH successfully by co-precipitation technique. This work focus on the structure and the stability of a synthesiz

ed drug–LDHs composite intended for providing basic data for organic–inorganic LDH hybrids.

Materials and Methods

Table (1): Chemicals used in the study:

Chemicals	Formula	Supplier
Aluminum Nitrate	$Al(NO_3)_3$	B.D.H (UK)
Ferric Chloride anhydrous	$FeCl_3$	B.D.H (UK)
Ferrous Sulphate Heptahydrate	$FeSO_4.7H_2O$	Scharlau (Germany)
Hydrochloric Acid	HCl	B.D.H (UK)
Liraglutide	$C_{172}H_{265}N_{43}O_{51}$	Novo Nordisk Denmark
Nickel Nitrate	$Ni(NO_3)_2$	B.D.H (UK)
Sodium Hydroxide	NaOH	B.D.H (UK)

Table (2): Instruments used in the study:

Instrument	Model	Supplier
Atomic force microscopy	AA3000.	Germany
Electrical balance	LC HD	DIGI China
Fourier Transform Infra-Red Spectroscopy (FTIR)	8400S	Shimadzu Japan
Hot plate magnetic stirrer	HPL-500-050M-220V	Gallenkamp England
pH Meter	WTW-INO LAB	Switzerland
Powder X-Ray Diffraction PXRD	XRD 6000 220V/50Hz	Shimadzu Japan
Scanning Electron Microscopy (SEM)	VEGA3LMU	Tescan Czech Republic
Zeta potential analyzer	Brookhaven	USA

Preparation of Fe^{+2}/Al^{+3} Layered Double Hydroxides nanoparticles

In the present work Fe^{+2}/Al^{+3} LDHs NPs was prepared by mixing 25 mL of 0.06 M of $Al(NO_3)_{3\ solution}$ and 25 mL of 0.02 M of $FeSO_4$ (3:1 ratio) respectively. To which 2.1 mL of 0.5 N HCl was added, then titrated drop by drop with 2 M NaOH with vigorous magnetic stirring at 80˚C until the pH elevated from 2 to 9 with change in the

color of the solution from clear yellow to light brown suspension which is then left at room temperature for 24 hours. The resultant suspension filtered and washed with deionized water several times, until the filtrate became neutral [5].

Preparation of Fe^{+2}/Al^{+3} Layered Double Hydroxides loaded liraglutide

The method used to prepare the liraglutide loading with LDH can be describe as: A solution containing 10 ml (0.0017g, 0.0008M) of Al $(NO_3)_3$ was added to a solution of 10 ml of (0.00064g, 0.0004M) $FeSO_4$. The mixture was treated with 1ml of liraglutide (0.006g, 0.0016 M) and repeated above procedure [6].

Preparation of Ni^{+2}/Fe^{+3} Layered Double Hydroxides nanoparticles

Ni^{+2}/Fe^{+3} LDHs NPs was prepared by mixing 25 mL of 0.75 M of Ni $(NO_3)_2$ solution and 25 mL of 0.25 M of $FeCl_3$ (3:1 ratio) respectively and repeated the same method that mentioned above [7].

Preparation of Ni^{+2}/Fe^{+3} Layered Double Hydroxides loaded liraglutide

Liraglutide loading with LDH was prepared also by same method and same procedure as above: A solution containing 10 ml (0.00146g, 0.0008M) of Ni $(NO_3)_2$ mixed with a solution of 10 mL (0.00064g, 0.0004M) of $FeCl_3$. Then the mixture was treated with 1ml of liraglutide (0.006g, 0.0016 M) [8].

Result and Discussion

FT-IR Spectra for Fe^{+2}/Al^{+3} LDH and Fe^{+2}/Al^{+3} LDH –loaded liraglutide

The FT-IR spectrum for Fe^{+2}/Al^{+3} LDH Figure 1 (a) show the bands at (3553, 3470 and 3371 cm^{-1}) assigned to the hydroxyl stretching (O-H) stretching and the band (1496 cm^{-1}) attributed to (O-H) bending. While the FT-IR spectrum for Fe^{+2}/Al^{+3} LDH loaded liraglutide Figure 1 (b) displays the broad bands at (3607and 3510 cm^{-1}) due to the hydroxyl stretching (O-H) stretching, while the bands at (3398 cm^{-1}) can be assigned for the (N-H) stretching of amide group, the NH2 group appears the stretching as a symmetrical and symmetrical groups at (3275 cm^{-1}) and the band at (1635 cm^{-1}) assigned to (C=C). That is refer to the physical binding between the liraglutige drug molecules and LDH by hydrogen bonds only [9].

FT-IR Spectra for Ni^{+2}/Fe^{+3} LDH and Ni^{+2}/Fe^{+3} LDH –loaded liraglutide

The FT-IR spectrum for Ni^{+2}/Fe^{+3} LDH figure 2 (a) shows the bands at (3470, 3444 and 3414 cm^{-1}) assigned to the hydroxyl stretching (O-H) stretching and the band (1541 cm^{-1}) attributed to (O-H) bending. While the FT-IR spectrum for Ni^{+2}/Fe^{+3} LDH loaded liraglutide figure 2 (b) displays the broad bands at (3514 and 3419 cm^{-1}) due to the hydroxyl stretching (O-H) stretching, while the bands at (3385 cm^{-1}) can be assigned for the (N-H) stretching of amide group, the band at (2069 cm^{-1}) due to the (C=N) stretching, the band (1790 cm-1) assigned to the carbonyl groups, the bands at (1635 and 1641 cm^{-1}) assigned to (C=C) and the band (1093 cm-1) due to (C-O) stretching band.

That is refer to the binding of Ni^{+2}/Fe^{+3} LDH with drug molecules as a hydrogen bond only.

In the other hand as the fingerprint there is no two unique molecular structures produce the same infrared spectrum so there are changes in the bands of prepared compounds before and after loaded liraglutide that because intercalation of drug [10].

X-ray diffraction (X-RD)

A general analysis of these patterns shows that the LDHs loaded liraglutise Figures (3 and 4) show the peaks at 33 $(2\ \Theta)$ assigned to the dihedral geometry as amorphous structure with small difference between LDHs types. Such difference is

attributed to the higher similarity of ionic radii for the ion pair [11].

Atomic force microscopy (AFM) Results:

Particle size measurements by AFM showed that particle size for Fe^{+2}/Al^{+3} LDH was 86.64 nm and for Ni^{+2}/Fe^{+3} LDHs was 53.19 nm and that size increased after loading with liraglutide to become 103.26 nm and 73.06 nm for $Fe^{+2}/Al^{+3}$and Ni^{+2}/Fe^{+3} LDHs-loaded liraglutide respectively.

Figures (5 and 6) showed the AFM images of the LDHs typical surface (in three and two dimensions) for LDHs before and after loading liraglutide.

Zeta potential measurement

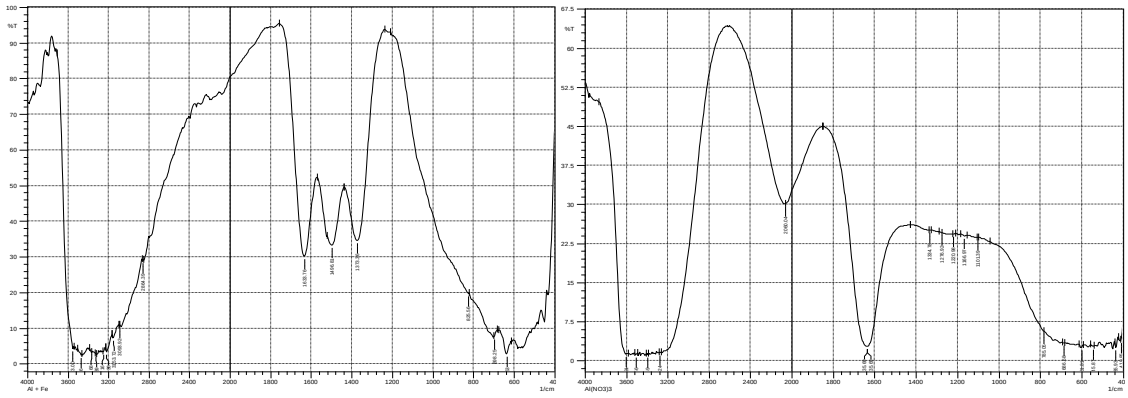
Zeta potential measurement for the prepared LDHs loaded liraglutide, Figures (7 and 8). The values of the zeta potential of the prepared compounds are listed in table 3, provided satisfactory evidence about their little tendency towards aggregation when its zeta potential in the negative scale and below -20 mV. These results suggested that the prepared LDHs loaded drug particles were stable with no tendency to aggregates and this in agreement with the results reported for colloidal nanoparticles behavior [12].

Table (3): Zeta potential values for the prepared LDHs NPs loaded liraglutide

LDHs NPs Type	Zeta Potential (mV) ± SD	Mobility(µ/s)/(V/cm) ±SD
Fe^{+2}/Fe^{+3} LDHs loaded liraglutide	-32.82	-2.56
Al^{+3}/Fe^{+2} LDHs loaded liraglutide	-26.45	-2.07
Ni^{+2}/Fe^{+3} LDHs loaded liraglutide	-28.00	-2.19

Scanning electron microscopy (SEM)

The pictures shown in Figures (9 and 10) represented the SEM images of LDHs loaded liraglutide. The images confirmed the layered shape of LDHS with multiple agglomeration areas, due to the hydrogen bonding attraction between multiple hydroxyl groups those had not been hydrolyzed by acidic hydrolysis [13,14].



a **b**

Figure (1): a-FT-IR spectrum of Fe^{+2}/Al^{+3} LDH, b- FT-IR spectrum of Fe^{+2}/Al^{+3} LDH loaded liraglutide.

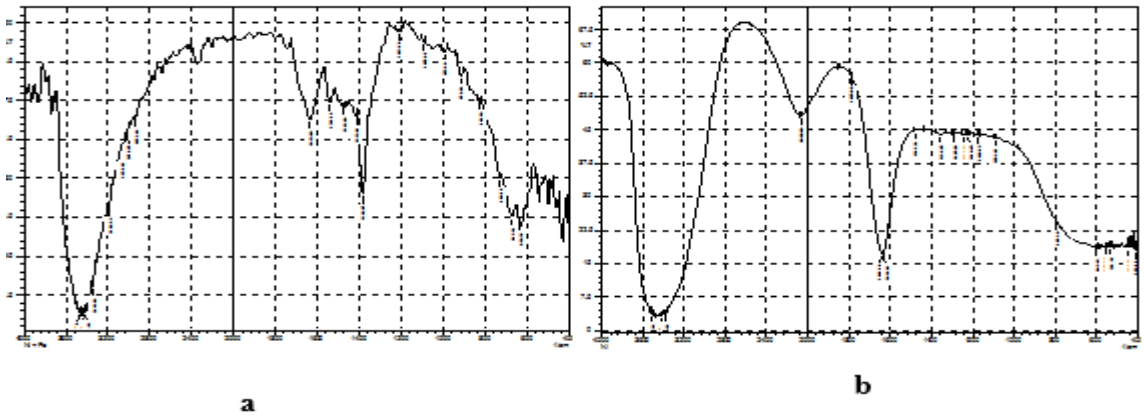


Figure (2): a- IR spectrum of Ni+2/Fe+3 LDH, b-FT-IR spectrum of Ni+2/Fe+3 LDH loaded liraglutide.

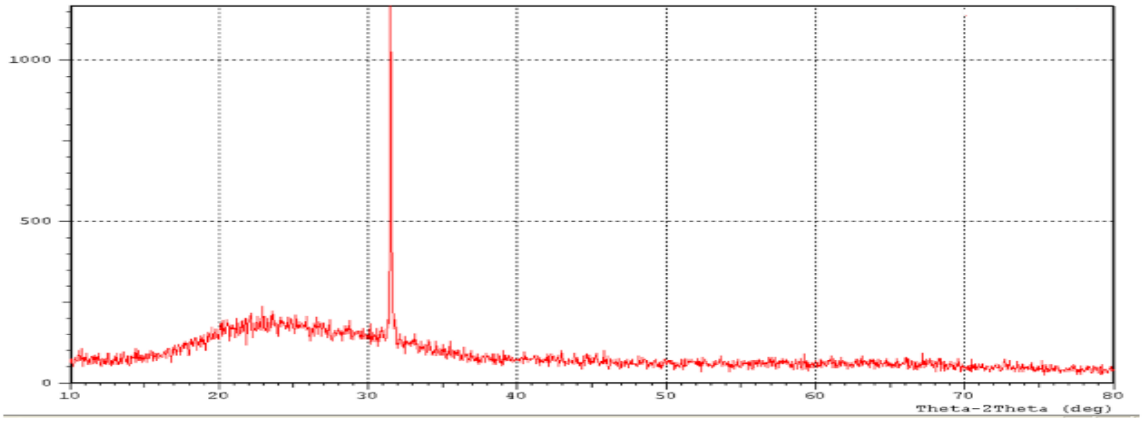


Figure (3): XRD pattern of Fe^{+2}/Al^{+3} loaded Liraglutide

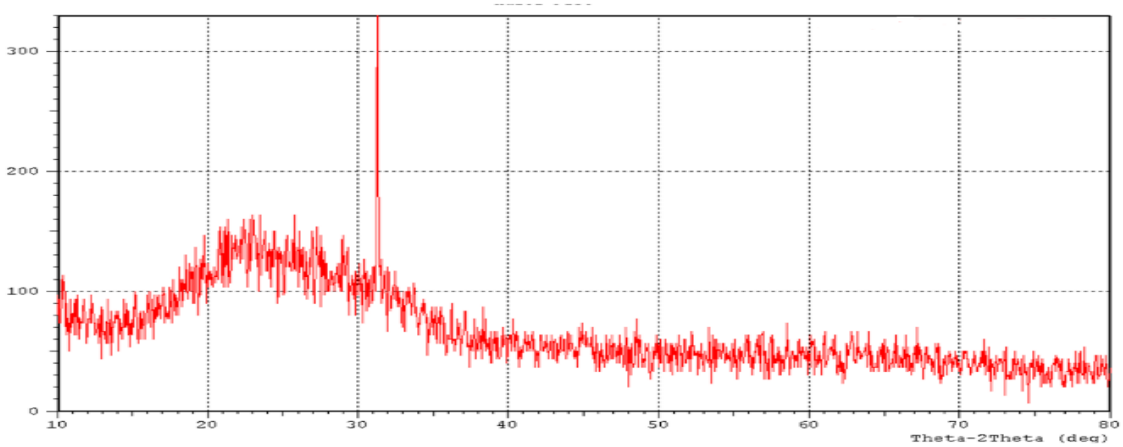


Figure (4): XRD pattern of Ni^{+2}/Fe^{+3} loaded liraglutide.

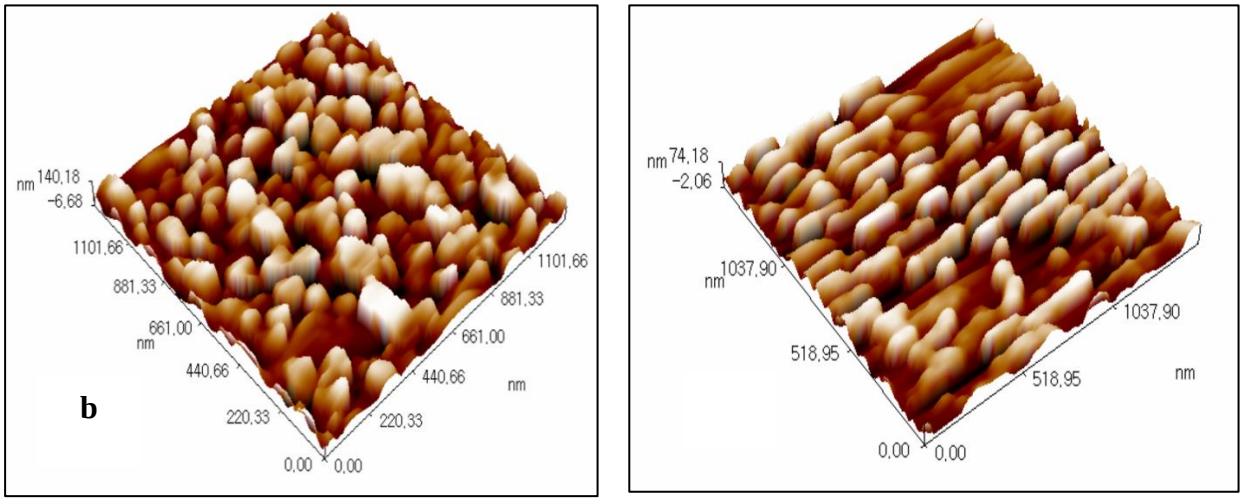


Figure (5): AFM images describing the granularity of (a) Fe+2/Al+3 LDHs and (b) Fe+2/Al+3 LDHs loaded liraglutide in three and two dimensions.

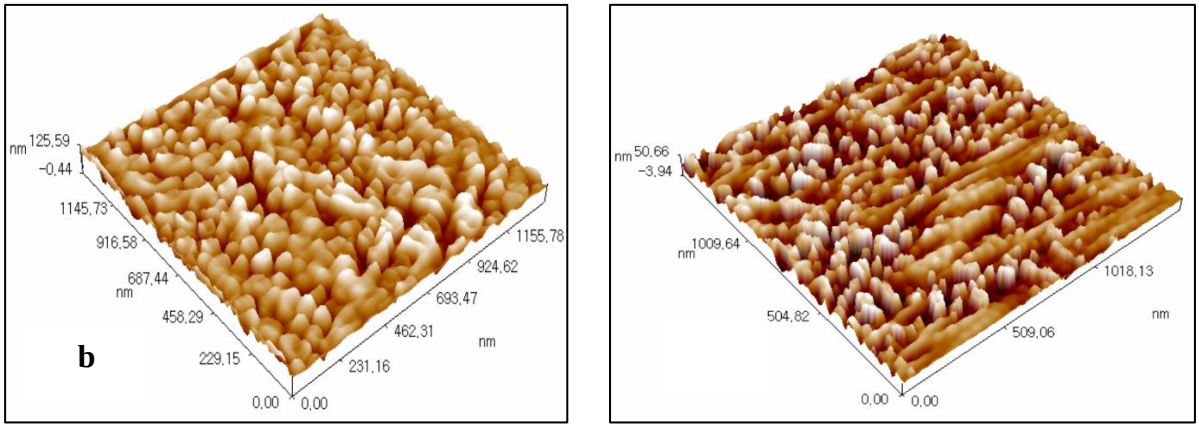


Figure (6): AFM images describing the granularity of (a) Ni+2/Fe+3 LDHs and (b) Ni+2/Fe+3 LDHs loaded liraglutide in three and two dimensions

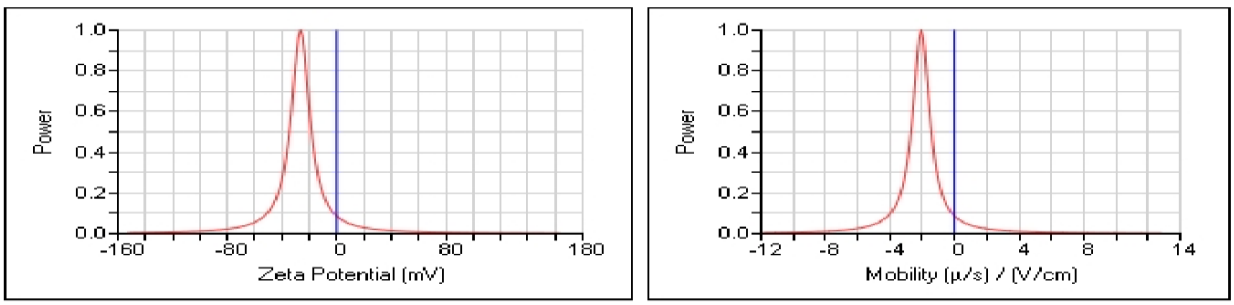


Figure (7): Zeta potential and mobility monographs of the prepared Al+3/Fe +2 LDHs loaded liraglutide.

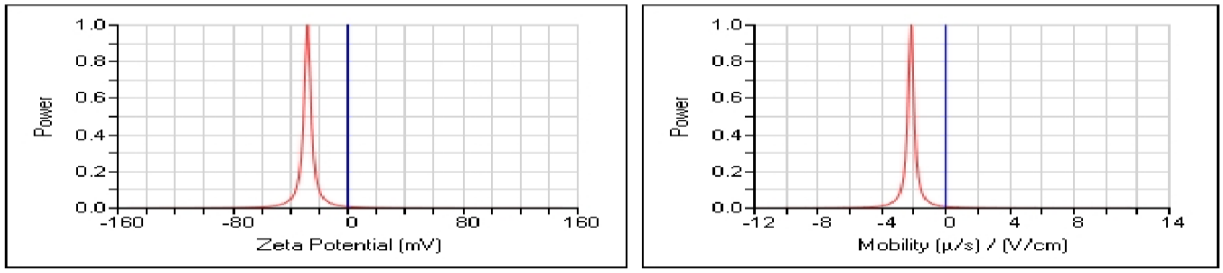


Figure (8): Zeta potential and mobility monographs of the prepared Ni+2/Fe+3 LDHs loaded liraglutide.

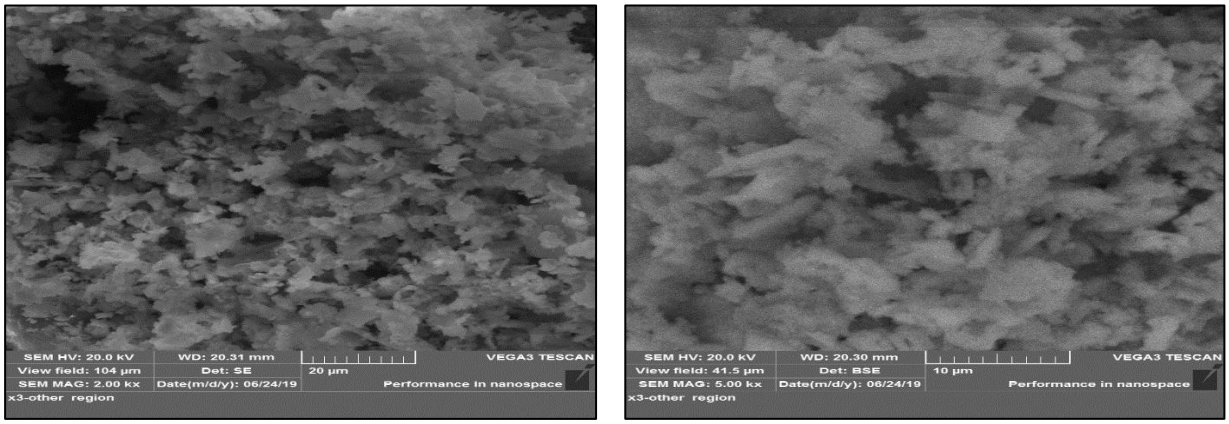


Figure (9): SEM image of the prepared Fe+2 / Al+3 LDHs loaded liraglutide in different measurements.

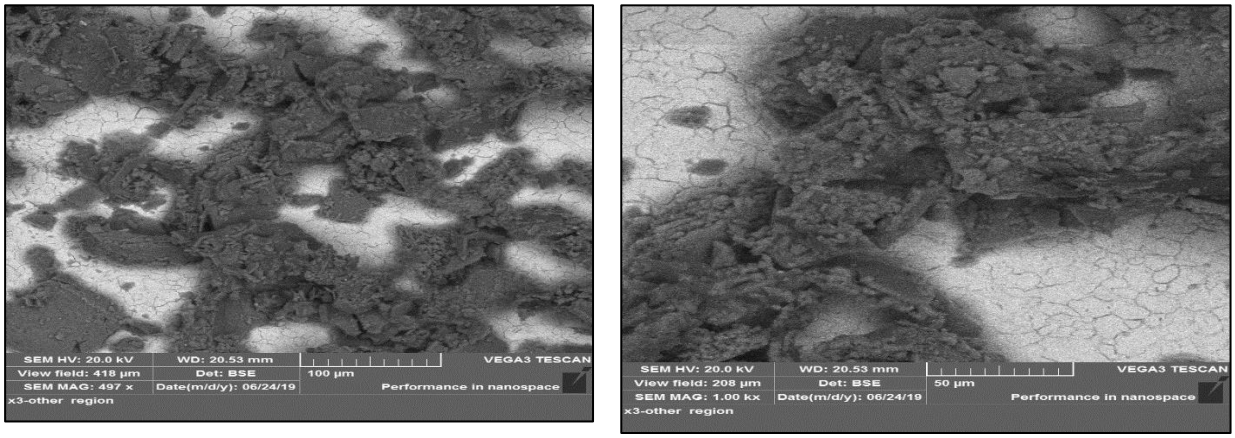


Figure (10): SEM image of the prepared Ni+2 / Fe+3 LDHs loaded liraglutide in different measurements.

Conclusion:

1. Intercalation of liraglutide drug with LDHs NPs via coprecipitation method provide a promising nanocarriers for control drug release and site-specificity.
2. Type of binding between nanocarrier and drug molecules was confirmed by FT-IR spectroscopy.

3. The size and layered shape of the nanocarrier were successfully confirmed by AFM and SEM.
4. Amorphous structure of prepared compound was confirmed by XRD.
5. The zeta potential analysis provided a satisfactory evidence about the prepared LDHs little tendency towards aggregation.

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