

Inclusion of L-Alanine, L-Arginine, L-Asparagine, L-Serine and Proline into θ -Type Zirconium Phosphate

Sadek Shakshooki, Abdurrahman Rhuma, and Fadel Siala

Abstract—Layered θ -Type zirconium phosphate (θ -Zr(HPO₄)₂·1.88H₂O) was prepared by the reaction of zirconium oxychloride in HF solution with dilute H₃PO₄ at 15°C. The resulting compound was intercalated with amino acids L-alanine, L-arginine, L-asparagine, L-serine and proline respectively at room and 600C temperatures. The structure properties of the obtained products were characterized by fourier transform infrared spectroscopy (FT-IR), X-ray powder diffraction (XRD) and thermal analysis (TG/DTA). FT-IR spectrum results demonstrated that the effectiveness of the vibrational spectra in characterizing of the resultant product was supported by TG/DTA and X-ray analysis. Moreover, the vibrational spectroscopic investigation shows that water molecules were appeared very characteristic infrared bands that can be assigned to regular hydrogen bonding of each molecule to adjacent OH group of (POH). Furthermore, amino acids inclusion products were found to retain the layered structure and the loading are closely dependent on the nature of the amino acid.

Index Terms—Inclusion, amino acids, θ -type zirconium phosphate.

I. INTRODUCTION

Tetravalent metal phosphates are very insoluble compounds which were known as amorphous for some time [1], [2]. The discovery of their crystalline layered structure by Clearfield and Stynes in 1964 [3] represents the fundamental step in the chemistry of these compounds and found to be α -layer of type. Their layered crystalline materials resemblance clay minerals [4]. The layered materials receiving great attention because of their size, structure, and possible biochemical applications [5] that have been proven to be good carriers for organic polar molecules. Examples of these are zirconium phosphates [4] taking advantage of the expandable interlayer space of the layered materials. Researchers have been capable of encapsulating functional biomolecules into these inorganic matrices protecting them from interacting with environment, avoiding denaturation and enhancing their shelf [4]-[6].

Porous layered M^{IV} phosphates with α , θ and γ -structures [7]-[9]. α -M^{IV}(HPO₄)₂·H₂O, θ -M^{IV}(HPO₄)₂·5H₂O and γ -M^{IV}·PO₄·H₂PO₄·2H₂O are well known compounds, (where M = Ti, Zr, Hf, Sn, Ce). These materials contain structural POH

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The authors are with the Department of Chemistry, Faculty of Science, University of Tripoli, UK (e-mail: shakshooki2002@yahoo.com, Rahuma1@hotmail.com).

groups with labile protons and therefore potential candidates as Intercalates [10], ionic co-conductance materials [11], solid acid catalysts [12] and sensors [13].

θ -zirconium phosphate, Zr(HPO₄)₂·5H₂O, has an interlayer spacing of 10.4 Å is readily prepared from protonation of its parent monosodium form Zr(NaPO₄)(PO₄)·5H₂O or from hydration of the metastable alcohol form Zr(HPO₄)₂·(C₂H₅OH)₂ [1], [14]. θ -zirconium phosphate Zr(HPO₄)₂·5H₂O, has a tendency to exchange large size metal ions, such as Cs⁺, Mg²⁺, Ba²⁺, divalent and trivalent transition metal ions and to intercalate bulky amines [1], [8].

Amino acids are biologically important organic compounds made from amine (-NH₂) and carboxylic acid (-COOH) functional groups, along with a side-chain specific to each amino acid. The key elements of an amino acid are carbon, hydrogen, oxygen, and nitrogen, though other elements are found in the side-chains of certain amino acids.

To date only few reports on intercalation of amino acids into α , or γ -layered M^{IV} phosphates [15]-[18] are available. In this study the synthesis and characterization of novel θ -type zirconium phosphate amino acids intercalated products were investigated.

II. MATERIALS AND METHODS

A. Chemicals

ZrOCl₂·8H₂O, H₃PO₄ (85 %) and amino acids L- Alanine C₃H₇NO₂, L-Asparagine C₄H₈N₂O₃, L-Proline C₅H₉NO₂, L-Serine C₃H₅O₃N and L-Lysine C₆H₁₄N₂O₂ used are of BDH, hydrofluoric acid HF (40%) of Reidel De-Haen. Other reagents used were of analytical grade.

B. Instruments Used for Characterization

X-Ray powder diffractometer Siemens D-500 was applied by using Ni-filtered CuK_α ($\lambda = 1.54056 \text{ \AA}$). TG/DTA SII Extra 6000 Thermogram and TG/DTA Perkin-Elmer SII were used to identify the thermal behavior of the obtained compounds. Fourier Transform FT-IR spectrometer was carried on FT/IR-600. All obtained spectra were measured within a spectral range of 4000–400 cm⁻¹. pH Meter GWG 521 were also used in this study to estimate the ion exchange properties of θ -Zr(HPO₄)₂·1.88H₂O. The investigation of C, H and N contents were analyzed with GMBH Vario Elemental Analyzer.

C. Preparation of θ -Zr (HPO₄)₂·1.88H₂O

50ml of 0.5M ZrOCl₂·8H₂O in 3M HF was added into Pyrex round bottom flask containing 200ml of (4.6M) H₃PO₄ (prior to mixing, the solutions were cooled at ~15°C). The

mixture prepared was kept under constant magnetic stirring at $\sim 15^\circ\text{C}$ for 3 days. The resultant precipitate was then washed with distilled water by addition and decantation of distilled water in order to obtain the pH 3 of the solution, then filtered and dried in the air.

D. Exchange Capacity Determination

Exchange capacity was determined by addition of 25 ml of 0.10 M NaCl solution to 100 mg of the $\theta\text{-Zr}(\text{HPO}_4)_2 \cdot 1.88\text{H}_2\text{O}$, with constant continuous stirring for 24 h, then titrated with 0.10 M of NaOH solution.

E. Inclusion of Amino Acids

Inclusion procedure was performed by addition of 0.1 M aqueous solutions of amino acids L-alanine, L-arginine, L-serine, L-lysine, L-asparagine and proline, respectively, into $\theta\text{-Zr}(\text{HPO}_4)_2 \cdot 1.88\text{H}_2\text{O}$.

Typically; to 0.25 g of $\theta\text{-Zr}(\text{HPO}_4)_2 \cdot 1.88\text{H}_2\text{O}$, 20 ml of 0.1 M L-serine were added at room temperature $\sim 25^\circ\text{C}$ and kept for 72 h with constant continuous stirring. In order to obtain the best intercalation condition for this guest molecule (same reaction repeated at 60°C for 5 h). The resultant product was filtered, washed with distilled water and dried in the air. Similar intercalation procedure was carried out with L-alanine, L-arginine, L-asparagine and proline. The products were designed as compounds I-V.

III. RESULTS AND DISCUSSION

θ -type zirconium phosphate, $\theta\text{-Zr}(\text{HPO}_4)_2 \cdot 1.88\text{H}_2\text{O}$, was prepared and characterized by chemical, X-ray, thermal analysis and by FT-IR spectroscopy. The exchange capacity was determined by Na^+ ions titration.

A. XRD Pattern of $\theta\text{-Zr}(\text{HPO}_4)_2 \cdot 1.88\text{H}_2\text{O}$

Data presented in Fig. 1 shows the X-ray powder diffraction pattern of the θ -type $\text{Zr}(\text{HPO}_4)_2 \cdot 1.88\text{H}_2\text{O}$, exhibit the presence of diffraction maxima with basal spacing equal to 9.85\AA and also exhibit lamellar structure. Negatively charged layers are formed by macroanions $[\text{M}_n(\text{IV})(\text{HPO}_4)_{2n}]^{2n-}$ and protons (H^+) bonded to the oxygen adjacent to the anionic layer form positively charged layers. However, the water molecules occupying crystallographic sites are located almost in the center of interlayer cavities.

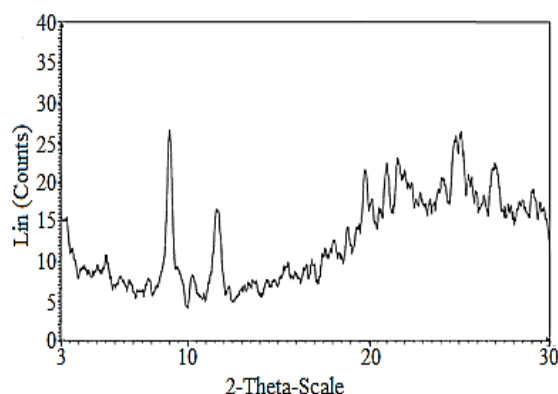


Fig. 1. X-ray diffractogram of $\theta\text{-Zr}(\text{HPO}_4)_2 \cdot 1.88\text{H}_2\text{O}$.

B. FT-IR Spectra of the $\theta\text{-Zr}(\text{HPO}_4)_2 \cdot 1.88\text{H}_2\text{O}$

FT-IR becomes a key tool to investigate structure. Fig. 2

show FT-IR spectra of $\theta\text{-Zr}(\text{HPO}_4)_2 \cdot 1.88\text{H}_2\text{O}$ in the range $4000\text{-}400\text{cm}^{-1}$ wave number.

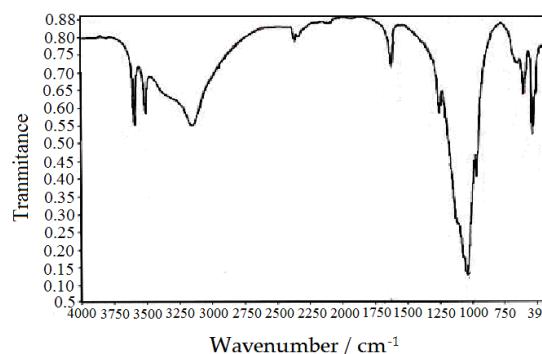


Fig. 2. FT-IR-Spectra of θ -type of $\text{Zr}(\text{HPO}_4)_2 \cdot 1.88\text{H}_2\text{O}$.

A tentative assignment of various vibrational 3 modes is proposed based on previous works performed in other M(IV) phosphate compounds [18].

The FT-IR spectra of $\theta\text{-Zr}(\text{HPO}_4)_2 \cdot 1.88\text{H}_2\text{O}$ show the same trend of modes. From Fig. 2, the narrow bands at 3604.65cm^{-1} , 3434.11 and band at 1640.39cm^{-1} are assigned to vibrational modes of H_2O molecules suggest that water molecules are located at well defined crystallographic sites. These bands at 3604.65cm^{-1} and 3434.11cm^{-1} were also attributed to an O-H asymmetric modes of interlayer water molecules. The band at 1640.39cm^{-1} also corresponds to H-O-H bending modes and the broad band at 3147.10cm^{-1} assigned to (P) OH stretching mode of the hydrogen bond, it had shoulder at 3310cm^{-1} attributed to O-H stretching coming from symmetry lowering effect of the H_2O interlayer molecules.

At the region $1273.21\text{-}1054.46\text{cm}^{-1}$, the bands are assigned as P-O asymmetry stretching of PO_4 groups, while at 976.33cm^{-1} is characteristic to the bonding in plane of the (P-O) bond.

In the region $609.14\text{-}515.39\text{cm}^{-1}$, the bands ascribed to the presence of $\delta(\text{PO}_4)$ and to vibrational of water molecules (609.14cm^{-1}), while at 671.64cm^{-1} is connected with O-H bond (out of plane).

C. Exchange Capacity

Exchange capacity of $\theta\text{-Zr}(\text{HPO}_4)_2 \cdot 1.88\text{H}_2\text{O}$ was determined by Na^+ ions titration. The titration curve is shown in Fig. 3. The exchange capacity found to be equal to 6.2Meq/g . The calculated value is 6.01Meq/g . This difference is due to partial hydrolysis of HPO_4^{2-} groups due to pH effect.

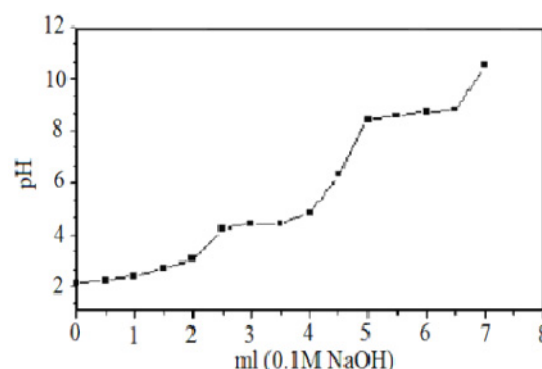


Fig. 3. Na^+ titration curve of θ -type of $\text{Zr}(\text{HPO}_4)_2 \cdot 1.88\text{H}_2\text{O}$.

D. TG/DTA of θ -Zr(HPO₄)₂·1.88H₂O

Thermal analysis of θ -type Zr(HPO₄)₂·1.88H₂O is shown in Fig. 4 and was carried out at temperature range ~25-800°C, in air atmosphere, the heating rate was 10°C/min. The thermal decomposition exhibits two weight loss stages, the loss of water of hydration followed by POH groups condensation.

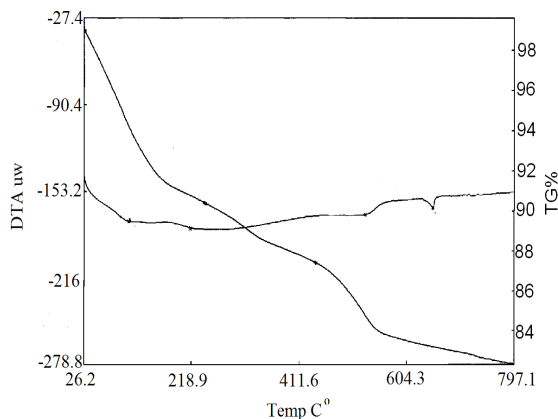


Fig. 4. TG/DTA pattern of θ -type of Zr(HPO₄)₂·1.88H₂O.

The first stage is accompanied by weight losses equal to 16.52% up to 210°C which have been attributed to the loss of water of hydration. The second stage in the region 320-650°C ascribed to the loss of the structure water due to POH groups condensation. The weight losses were 5.65% and final product was the pyrophosphates ZrP₂O₇.

Amino acids L-alanine, L-asparagine, L-serine, L-arginine, and proline based θ -Zr(HPO₄)₂·1.88H₂O have been synthesized at room and at 60°C temperatures. The Loading of the amino acids at 60°C found to be less than 30% for L-arginine, θ -Zr(HPO₄)₂(arg)_{1.32}·H₂O almost the same for L-proline θ -Zr(HPO₄)₂(prol)_{0.02}·1.1H₂O and low for L-asparagine θ -Zr(HPO₄)₂(aspg)_{1.32}·H₂O and L-serine θ -Zr(HPO₄)₂(ser)_{0.39}·0.5H₂O. However, it was no uptake in the case of L-analine.

Their elemental contents of C, H, N at room temperature have been characterized by elemental (C, H, N) and TGA analysis and the results are shown in Table I. The loading of the amino acid found to be dependent on its nature. The resultant amino acids inclusion products were formulated from combination of elemental and thermal analysis and were designated as compounds (I-V) and their water of hydration were calculated from the TG curve, Fig. 5-Fig. 8.

TABLE I: PERCENT ELEMENTS CONTENT (C, N, H) OF AMINO ACIDS ZIRCONIUM PHOSPHATE COMPOUNDS (I-VI)

Compound	C	H	N
(I) θ -Zr(HPO ₄) ₂ (anal) _{0.0}	nil	nil	nil
(II) θ -Zr(HPO ₄) ₂ (aspg) _{0.54} ·1.63H ₂ O	10.6	2.0	6.18
(III) θ -Zr(HPO ₄) ₂ (ser) _{0.39} ·0.5H ₂ O	8.27	1.6	3.2
(IV) θ -Zr(HPO ₄) ₂ (arg) _{1.32} ·H ₂ O	22.8	4.78	15.97

E. TGA of Amino Acids Inclusion Products

The thermal analysis used in this and other studies found to be a good tool for characterization of organic inclusion products of M(IV) phosphates with good accuracy in the range of ~2% [5], [6], [19].

TG/DTA, of asparagine inclusion product θ -Zr(HPO₄)₂

(aspg)_{0.54}·1.63H₂O compound (II) is shown in (Fig. 5).

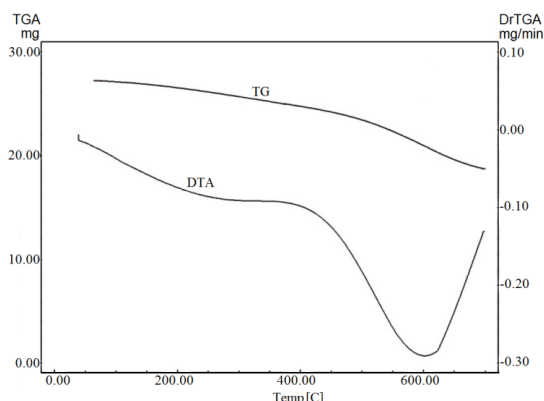


Fig. 5. TG/DTA pattern of θ -Zr(HPO₄)₂(aspg)_{1.32}·H₂O.

It can be seen that thermal decomposition found to occur in two stages. The loss of water of hydration initiated at ~65°C up to 160°C, followed by de-intercalation, decomposition of the organic compound and POH groups condensation. The thermal decomposition of the organic compound found to superimpose to that of the POH groups condensation. The weight loss was accompanied by endothermic broad peak centered at ~270°C. Total weight loss found to be 31.15%.

Fig. 6 shows TG /DTA of inclusion product, θ -Zr(HPO₄)₂(ser)_{0.39}·0.5H₂O, compound (III). The thermal decomposition found to be occurred in two stages. Compared with Fig. 5, the loss of water of hydration initiated at ~60°C up to 150°C and followed by de-intercalation, decomposition of the organic compound and POH groups condensation, this is mainly due to the POH groups condensation started at about 300 °C. The thermal decomposition of the organic compound found to superimpose to that of the POH groups condensation. The weight loss was accompanied by two endothermic broad peak centered at 200, 500°C. Total weight loss found to be 16.45%.

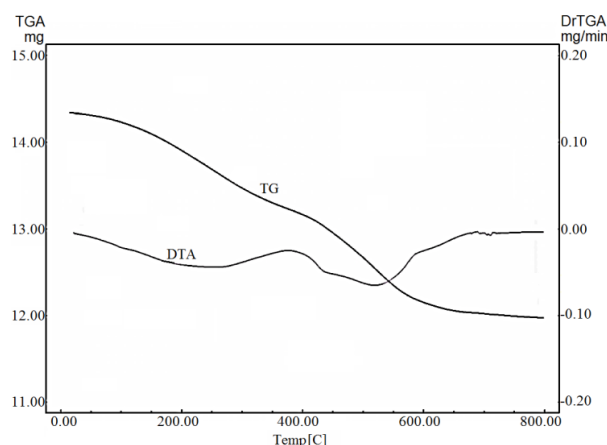


Fig. 6. TG/DTA pattern of θ -Zr(HPO₄)₂(ser)_{0.39}·0.5H₂O.

TG/DTA pattern of inclusion product, θ -Zr(HPO₄)₂(arg)_{1.32}·H₂O, compound (IV) is shown in Fig. 7. From the figure we can see that the loss of water of hydration initiated at ~60°C up to 150°C and followed by de-intercalation, decomposition of the organic compound and POH groups condensation. The thermal decomposition of the organic compound found to superimpose to that of the POH groups condensation. The weight loss was accompanied by broad endothermic peak centered at 270°C

which very similar to that of θ -Zr(HPO₄)₂ (aspg)_{0.54}. 1.63 H₂O. Total weight loss found to be 50.07% which higher than that of θ -Zr(HPO₄)₂ (aspg)_{0.54}. 1.63 H₂O and θ -Zr(HPO₄)₂ (ser)_{0.39}. 0.5H₂O.

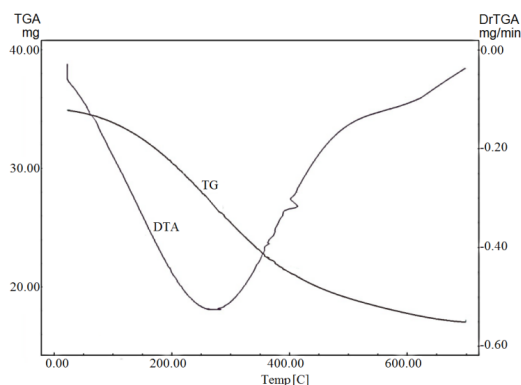


Fig. 7. TG/DTA pattern of θ -Zr(HPO₄)₂ (arg)_{1.32}. H₂O.

Fig. 8 shows TG/DTA pattern of inclusion product, θ -Zr(HPO₄)₂(prol)_{0.02}. 1.1H₂O compound (V), as shown in Fig. 8. The loss of water of hydration initiated at ~65°C upto 170°C and followed by de-intercalation, decomposition of the organic compound and POH groups condensation. The thermal decomposition of the organic compound found to superimpose to that of the POH groups condensation. The weight loss was accompanied by two broad endothermic peaks centered at 200,600°C. Total weight loss found to 13.04%.

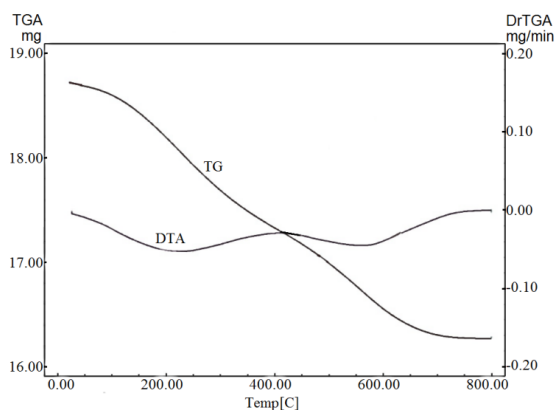


Fig. 8. TG/DTA pattern of θ -Zr(HPO₄)₂ (prol)_{0.02}. 1.1H₂O.

IV. CONCLUSION

The FT-IR spectra is a key tool to detect the presence of water molecules as well as to investigate the H-bonds in layered M(IV) phosphate. In this study, we reveal that θ -type zirconium phosphate has a tendency to intercalate basic amino acids with good loading. The loading found to be dependent on the nature of the amino acid and the lower loading of proline can be attributed to covering effect [1], [10]. The thermal behavior of the investigated materials found to follow the same trend of that observed for inclusion organic products of tetravalent metal phosphates. The C, H, N and TGA analysis were a good tool for the formulation of the end products. Future work will be carried on the protein, encapsulation via direct ion exchange with θ -type zirconium phosphate and the controlled release studies using pH changes.

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Sadek Shakshooki is a Ph.D. Currently he is a professor in Department of Chemistry, Faculty of Sciences, University of Tripoli, Libya.

In 1971, he obtained his Ph.D. in inorganic chemistry, Bristol School of Chemistry, Bristol University, Bristol, UK. In 1975-1978, He was the dean College of Pharmacy, University of Tripoli, Libya. In 2000, He awarded as outstanding staff member and researcher of Faculty of Science, from University of Tripoli; given for 1st time.

He has more than 140 publications in international journals, local and international conferences.

His research interest includes metal complexes, desalination, inorganic ion-exchangers of tetravalent metal phosphates, their pellicular membranes, intercalation, inclusion of some heterocyclic compounds into layered and glassy materials, proton conductance, inorganic membrane-membrane composites, pillared materials. Established three novel methods for crystallization of glassy and amorphous tetravalent metal phosphates to their layered materials. He established novel methods for pellicular membranes And fibrous membranes of tetravalent metal phosphate.



Abdurrahman M. Rhuma is a Ph.D. Currently he is an lecturer in Department of Chemistry, Faculty of Science, University of Tripoli, Libya,

In 2007, he obtained his Ph.D. in Biochemistry, in De Montfort University, Leceister, UK.. In 1984, he received his BSc in chemistry from University of Tripoli. From 2008 to date he is The Head of Chemistry Department, faculty of Education, University of Tripoli, Libya and the chief editor of

the journal of Education.

He has one recent publications: A. Rhuma, R. Jenkins, P. Haris, "Antimicrobial activities of *Nigella sativa* floral honey towards selected pathogenic and non-pathogenic microorganism," *J. of Ed.*, vol. 7, pp. 12-20.

His research interest includes bioactive compounds analysis, characterizing of antimicrobial and anticancer compounds. Inclusion of heterocyclic and organic compounds into layered materials and protein analysis.



Fadel Siala is a PhD. Currently he is an associate professor in Department of Chemistry, Faculty of Science, University of Tripoli, Libya.

In 1975, He obtained his PhD in Organic Chemistry. London University, London, UK. In 1987 and 1990 he was the head of Chemistry Departement, Faculty of Science, University of Tripoli, Libya.