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Green Synthesis of Cobalt Ferrite ($CoFe_2O_4$) Nanoparticles Utilising Co-Precipitation, Structural Features and Toxicological Evaluation against MCF-7 and HUVEC Cell Lines

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Abstract **Background:** Cobalt ferrite ($CoFe_2O_4$) nanoparticles were produced using an eco-friendly co-precipitation technique and their morphological, structural and cytotoxic properties were evaluated green synthesis approach to produce these nanoparticles. **Methods:** Physicochemical investigation was conducted using Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), energy-dispersive X-ray spectroscopy (EDX), scanning electron microscopy (SEM) and atomic force microscopy (AFM). X-ray diffraction (XRD). The cytotoxic activity of the cobalt and iron oxide nanoparticles was evaluated using the MTT assay on human umbilical vein endothelial cells (HUVEC) and breast cancer cells (MCF-7) after 24 and 48 hours of exposure at doses ranging from (25 - 400 µg/ml). **Results:** XRD confirmed a single-phase spinel structure with an average crystalline size of 20.17 nm, as estimated by the Debye-Scherer equation. The results showed a clear decrease in cell viability for both species, depending on the concentration and duration of exposure, with a significant reduction in IC50 values after prolonged exposure 147 µg/ml for HUVEC and 257 µg/ml for MCF-7 after 48 hours. **Conclusion:** These findings provide valuable insights into the biological response to iron oxide and cobalt nanoparticles and highlight the importance of assessing biosafety prior to their use in biomedical applications.

Key Words $CoFe_2O_4$ Nanoparticles, Green synthesis, Cytotoxicity, HUVEC, MCF-7

INTRODUCTION

Magnetic nanoparticles have garnered a lot of interest due to their unique size-dependent physical and chemical properties as well as their numerous applications in the fields of technology, medicine and the environment [1,2]. Among these materials, spinel ferrites, which have the general formula MFe_2O_4 (where M = Co, Ni, Zn and Mn), are especially intriguing due to their chemical stability, tuneable magnetic behaviour and well-defined crystal structure [3]. Cobalt ferrite can be used in targeted drug delivery and cancer treatment due to its strong crystalline magnetic contrast, mechanical durability and moderate magnetic saturation [4-6]. However, the increasing use of cobalt ferrite nanoparticles has raised concerns about potential adverse effects on cells and the environment. These nanoparticles can interact with biological systems through mechanisms such as oxidative stress, membrane rupture and the release of metal ions, depending on the particle size, the concentration of the surface chemical composition and the duration of exposure [7-9]. While endothelial cells such as HUVEC cells are commonly used to assess the biocompatibility of nanoparticles, breast cancer cell lines such as MCF-7 cells are commonly used to study antiproliferative effects [10,11]. This study used an environmentally friendly co-precipitation method to evaluate the structural, morphological and cytotoxic properties of $CoFe_2O_4$ nanoparticles for both normal and cancer cell lines under identical experimental conditions.

Objectives

The main objective of this study is to synthesize and characterize $CoFe_2O_4$ nanoparticles using an eco-friendly method and to evaluate their cytotoxic effects HUVEC and MCF-7 cell lines.

METHODS

Green Synthesis of $CoFe_2O_4$ Nanoparticles by Co-Precipitation

The green co-precipitation method, an effective and eco-friendly technique for spinel ferrites, was used to create $CoFe_2O_4$

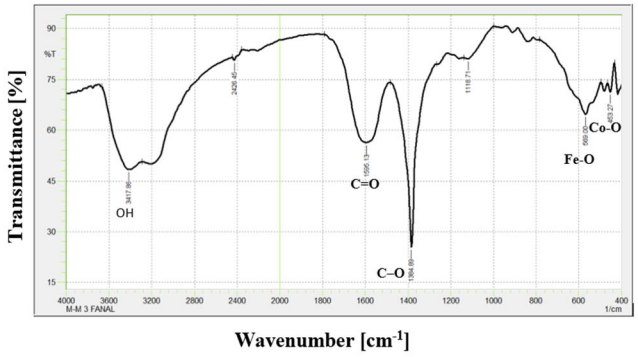

Figure 1: FTIR spectra of main salts and $CoFe_2O_4$ nanoparticles

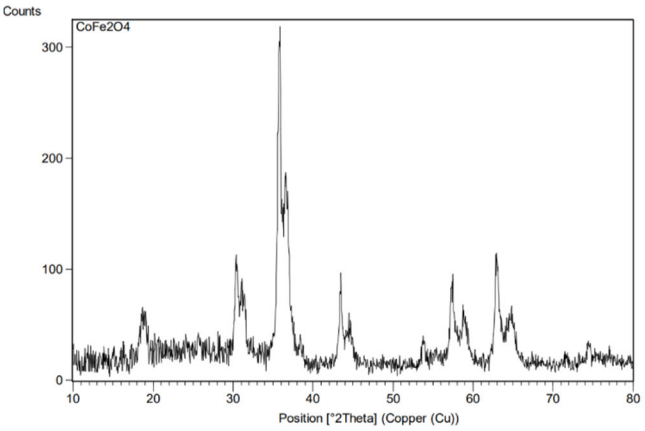

Figure 2: CoFe2O4 nanoparticles' X-ray diffraction pattern in comparison to ICDD standard data

Table 1: The Debye-Scherer equation was used to determine the crystal size of $CoFe_2O_4$ nanoparticles

Dp Average (nm)	Dp (nm)	FWHM B_{ae} (°)	Peak position 2θ (°)	λ (Å)	K
20.17	8.55	0.984	18.7852	1.54178	0.94
	29.15	0.2952	30.4039		
	14.60	0.5904	31.1387		
	22.17	0.3936	35.8091		
	17.78	0.492	36.6179		
	30.28	0.2952	43.4312		
	13.03	0.6888	44.5535		
	31.54	0.2952	53.8078		
	24.05	0.3936	57.3604		
	16.15	0.5904	58.8827		
	24.74	0.3936	62.9773		
	12.49	0.7872	64.7034		
	17.66	0.5904	74.4631		

nanoparticles [12,13]. In summary, 150 mL of deionised water was used to dissolve 1 g of cobalt nitrate hexahydrate (Co $(NO_3)_2 \cdot 6H_2O$) and 1 g of iron nitrate nicotinate (Fe $(NO_3)_3 \cdot 9H_2O$) with constant magnetic stirring at 25°C for 20 minutes. One gram of anhydrous citric acid was then added as a chelating agent. Ammonium hydroxide (NH_4OH) was added progressively to the reaction mixture until its pH reached 7.5. Cobalt ferrite nanoparticles were successfully co-precipitated when the mixture was heated to 135 degrees Celsius and a gelatinous precipitate developed.

Fourier Transform Infrared Spectroscopy (FTIR) of $CoFe_2O_4$

The functional groups and metal-oxygen linkages in the produced $CoFe_2O_4$ nanoparticles were investigated using Fourier transform infrared spectroscopy (FTIR). As illustrated in Figure1, the FTIR spectra of the produced sample were compared to those of $Co(NO_3)_2 \cdot 6H_2O$ and $Fe(NO_3)_3 \cdot 9H_2O$. The Co-O and Fe-O stretching vibrations, which are distinctive characteristics of the spinel ferrite structure, were identified as the cause of the unique absorption bands at roughly 438 cm^{-1} and 542 cm^{-1}, respectively [14]. The stretching vibrations of the C-O and C=O bonds connected to the leftover citric acid are responsible for the extra bands seen at 1384 cm^{-1} and 1596 cm^{-1}, while the stretching vibrations of the O-H bonds are responsible for the broad band about 3417 cm^{-1} [15].

X-Ray Diffraction (XRD) of $CoFe_2O_4$

As seen in Figure 2, X-ray diffraction analysis was used to examine the crystal structure of the produced $CoFe_2O_4$ nanoparticles. The creation of a single-phase spinel structure was confirmed by the close match between the diffraction peaks and the conventional spinel pattern of $CoFe_2O_4$ (ICDD card No. 22-1086) [16]. The Debye-Scherer equation was used to determine the average crystal size and Table 1 summarises the findings. The computed average crystal size of 20.17 nm demonstrated the synthesised material's nanocrystalline nature.

$$D = \frac{K\lambda}{\beta Cos\theta}$$

Energy - Dispersive X- ray (EDX) Spectroscopy of $CoFe_2O_4$

To ascertain the elemental makeup of the produced nanoparticles, EDX analysis was carried out. With weight percentages of 44.6% and 38.7%, respectively, the EDX spectrum displayed in Figure 3 verified the existence of iron and cobalt as the primary components, demonstrating the exceptional purity of the produced $CoFe_2O_4$ nanoparticles [17].

Scanning Electron Microscopy (SEM) of $CoFe_2O_4$

The surface morphology of the $CoFe_2O_4$ nanoparticles was investigated using SEM analysis. The particles were mostly in the nanoscale range, as seen in Figure 4 and partial agglomeration was ascribed to the electrostatic and magnetic interactions that are frequently seen in magnetic nanoparticles [18]. According to SEM scans, the average particle diameter was roughly 55.75 nm.

Atomic Force Microscopy (AFM) of $CoFe_2O_4$

The surface topography and roughness of the produced nanoparticles were thoroughly examined using atomic force microscope analysis. The average particle diameter was 124 nm, the average surface roughness (Sa. Roughness) was 178 picometres and the average root-mean-square roughness (Sq. Root mean square) was 471 picometres, according to the AFM data, which are displayed in Figure 5.

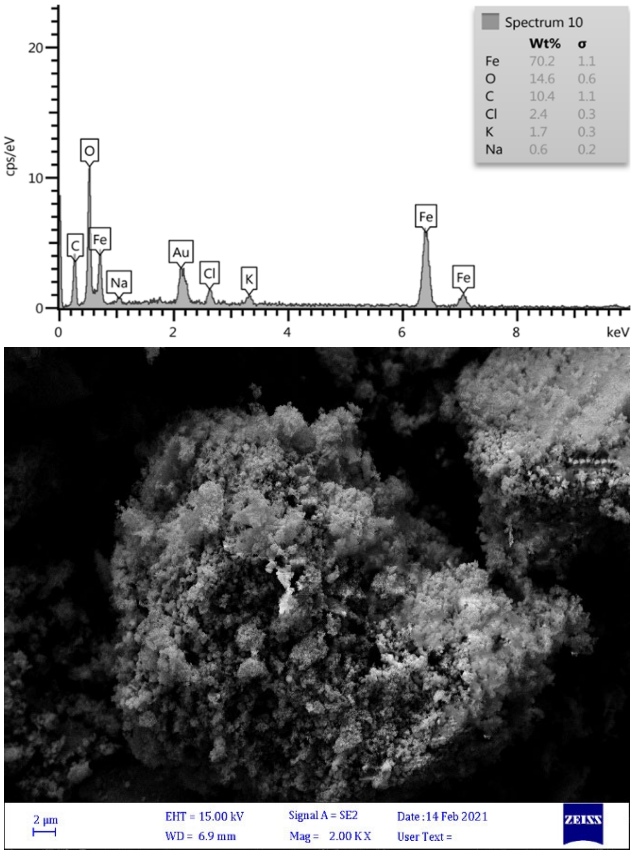

Figure 3: EDX spectrum showing the elemental composition of $CoFe_2O_4$ nanoparticles

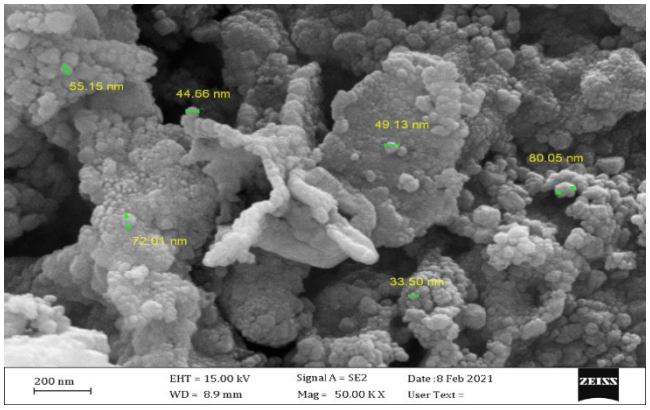

Figure 4: SEM micrographs of $CoFe_2O_4$ nanoparticles

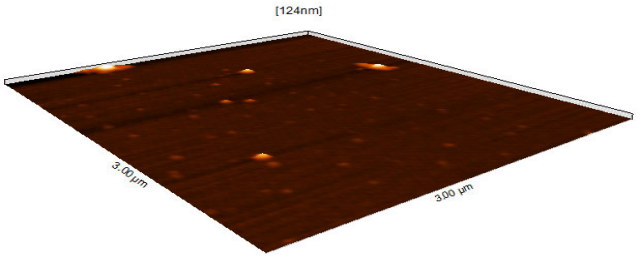

Figure 5: Atomic Force Microscopy Examination of $CoFe_2O_4$ Nanoparticles' Surface Topography and Roughness

Cytotoxicity Assay (MTT Test)

The MTT test, a well-used technique for evaluating cell viability based on mitochondrial metabolic activity, was used to investigate the cytotoxic effects of $CoFe_2O_4$ nanoparticles [19]. HUVEC and MCF-7 cells were exposed to $CoFe_2O_4$ nanoparticles at concentrations ranging from 25 to 400 µg/ml for 24 and 48 hours. Each experiment was performed three times and the results were expressed using the mean ± standard deviation.

RESULTS AND DISCUSSION

Cytotoxicity towards HUVEC Cells

After 24 hours of exposure, $CoFe_2O_4$ nanoparticles showed a concentration-proportional decrease in HUVEC cell viability. Cell viability exceeded 100% at a concentration of 25 µg/ml but decreased to 38.54% at a concentration of 400 µg/ml, as shown in (Table 2, Figure 6). Statistical analysis revealed a significant difference between concentrations ($P < 0.05$), with an IC50 value of 221 µg/ml (Figure 7). After 48 hours of exposure, a more pronounced cytotoxic effect was observed, with cell viability decreasing to 22.53% at a concentration of 400 µg/ml (Table 3, Figure 8). The IC_{50} value decreased to 147 µg/ml (Figure 9), indicating an increasing cytotoxic response over time.

Cytotoxicity of MCF-7 Cells

MCF-7 cells exhibited a clear cytotoxic reaction to $CoFe_2O_4$ nanoparticles, which was influenced by both concentration and duration. According to (Table 4 and Figure 10), cell

Table 2: Toxic effects of $CoFe_2O_4$ nanoparticles on HUVEC cells after 24 hours.

Concentration	R1%	R2%	R3%	Mean%	SD
0	95.6133	99.06304	105.3237	100	4.922536
25	106.6014	104.1738	104.4293	105.0682	1.33394
50	94.33561	89.99149	91.39694	91.90802	2.216698
100	72.23169	77.08689	80.15333	76.49064	3.994336
200	64.31006	55.87735	59.7104	59.96593	4.222158
400	42.71721	34.15673	38.75639	38.54345	4.28421

Table 3: Toxic effects of $CoFe_2O_4$ nanoparticles on HUVEC cells after 48 hours.

r	R1%	R2%	R3%	Mean%	SD
0	101.8288	98.83625	99.335	100	1.603267
25	93.84872	94.47216	98.33749	95.55279	2.431688
50	83.99834	84.24772	87.6143	85.28679	2.01954
100	59.18537	62.55196	56.56692	59.43475	3.000302
200	41.60433	36.6168	37.86368	38.69493	2.595594
400	19.40981	25.27016	22.90108	22.52702	2.948028

Table 4: Shows the harmful effects of $CoFe_2O_4$ nanoparticles on MCF-7 cells over a 24-hour period.

Concentration	R1%	R2%	R3%	Mean%	SD
0	100.0456	98.93628	101.0317	100.0045	1.048305
25	99.42931	96.71761	98.44324	98.19672	1.372554
50	93.38962	96.4711	97.45717	95.77263	2.121821
100	87.71971	91.91051	89.32208	89.65077	2.114649
200	71.69604	70.58671	73.05189	71.77822	1.234642
400	27.69259	24.48786	27.93911	26.71452	1.925367

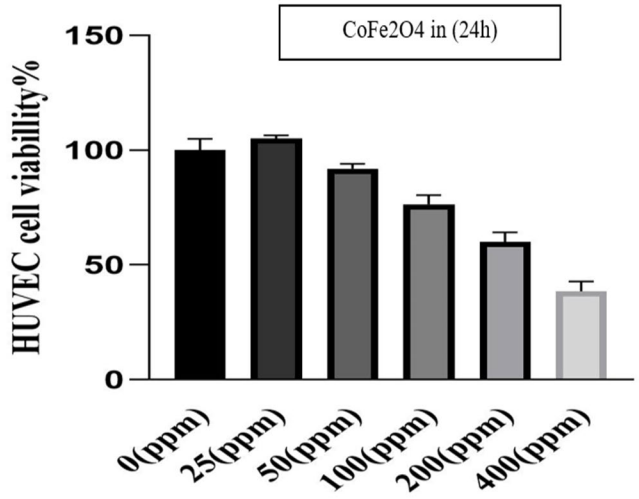

Figure 6: Cobalt ferrite (CoFe2O4) nanoparticles' 24-hour MTT test on HUVEC

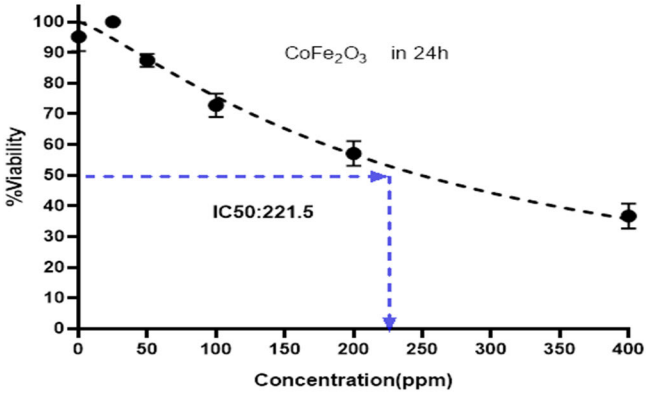

Figure 7: 24-hour IC50 for cobalt ferrite (CoFe2O4) nanoparticles

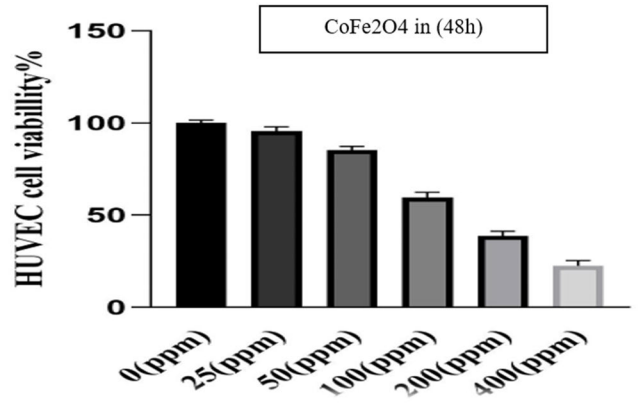

Figure 8: 48-hour MTT test of cobalt ferrite (CoFe2O4) nanoparticles on HUVEC

viability dropped to 26.71% at a dose of 400 µg/ml after a 24-hour exposure. After 24 hours, the IC50 for MCF-7 cells was determined to be 294.3 µg/ml (Figure 11). Cell viability dropped to 22.56% at the same concentration after 48 hours

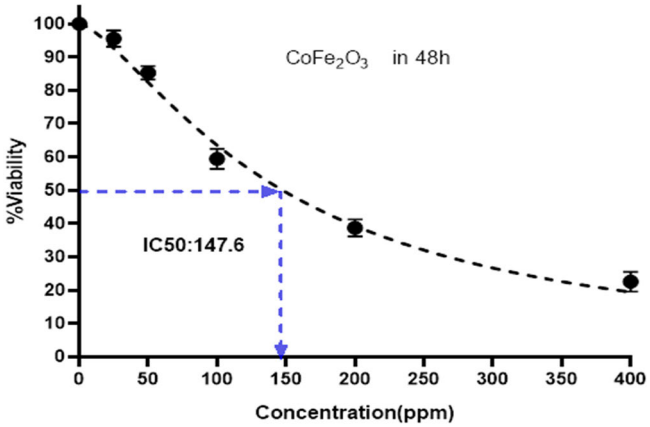

Figure 9: Half-inhibition concentration (IC50) of cobalt ferrite (CoFe2O4) nanoparticles after 48 hours

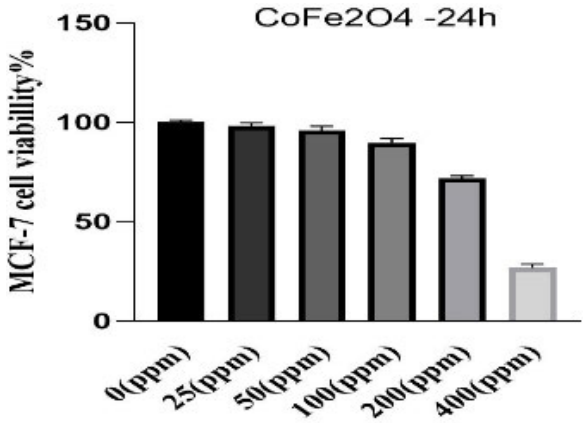

Figure 11: shows the 24-hour IC50 for cobalt ferrite (CoFe2O4) nanoparticles on MCF-7

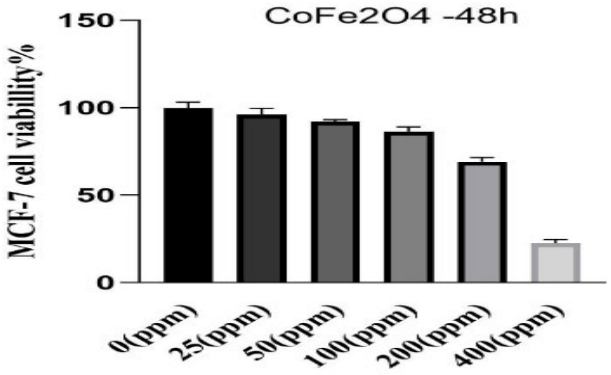

Figure 12: MTT test of cobalt ferrite (CoFe2O4) nanoparticles on MCF-7 in 48 hours

of treatment, indicating a more severe cytotoxic effect (Table 5, Figure 12). After 48 hours, the IC50 value dropped to 257.6 µg/ml (Figure 13), suggesting a growing cytotoxic effect. Overall, the findings demonstrate that MCF-7 cells were more sensitive to $CoFe_2O_4$ nanoparticles than HUVEC cells, suggesting that under equal experimental settings, cancer cells and normal cells respond differently.

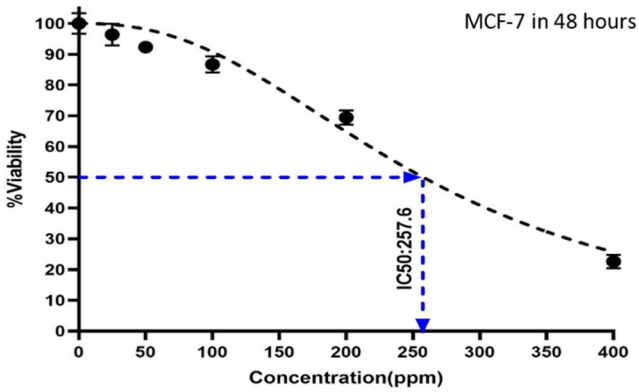

Figure 13: shows the IC50 of cobalt ferrite (CoFe2O4) nanoparticles on MCF-7 after 48 hours

Table 5: Shows the harmful effects of $CoFe_2O_4$ nanoparticles on MCF-7 cells over a period of 48 hours.

Concentration	R1%	R2%	R3%	Mean%	SD
0	98.81302	103.4969	96.96413	99.758	3.367324
25	93.1431	95.23851	100.0456	96.14241	3.538914
50	91.0477	93.26636	92.03377	92.11595	1.111611
100	89.32208	84.1452	85.99408	86.48712	2.623419
200	67.25872	68.61457	71.8193	69.23086	2.34192
400	20.42031	22.51572	24.73438	22.5568	2.157325

CONCLUSIONS

An environmentally acceptable co-precipitation approach was used to synthesize and systematically characterize cobalt-iron oxide ($CoFe_2O_4$) nanoparticles. While cytotoxicity experiments showed concentration- and time-dependent effects on both HUVEC and MCF-7 cell lines, structural investigations verified the creation of a crystalline nano-spinel phase. These results provide a strong experimental basis for further research on cobalt ferrite nanoparticles with a focus on their biosafety and potential biological applications.

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