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Synthesis of metal organic framework (MOF-5) embedded cryogel composite and its application for the extraction and determination of cholesterol from milk samples

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RESEARCH ARTICLE

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ABSTRACT

Metal-organic frameworks (MOFs)/coordination polymers (CPs) are a new class of hybrid inorganic/organic porous material. Recently MOFs have attracted much attention due to their large surface area, tunable pore structure, and high thermal stability. MOF-5 is composed of zinc salt and 1,4 benzene dicarboxylic linker. In the current study, MOF-5/cryogel composite was applied to extract cholesterol selectively from milk samples. This extraction process was used to clean-up milk samples with MOF-5/cryogel composite followed by determination of cholesterol in milk using UV-Vis spectrophotometric technique. The parameters such as concentration of cholesterol, volume of cholesterol solution, adsorbent amount, adsorption and desorption time were studied to obtain good extraction of cholesterol. The amount of cholesterol adsorbed and desorbed was 84 and 80%, respectively, from milk samples using MOF-5 composite cryogel. The developed method was validated in terms of linearity, accuracy, precision, limit of detection, and quantification. The response was linear in the range of 5-200 µg/mL with a coefficient of determination (r^2) of 0.990. Detection limit (0.15 µg/mL) and quantification (0.45 µg/mL) were obtained.

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1. Introduction

Cholesterol (CHO) is the major part of animal lipid sterols and found in vegetable oils [1]. It is a precursor of vitamin D, steroids, hormones, and bile acids. Most of the cardiovascular diseases are related with increased amount of CHO. In addition, human disorders such as Alzheimer's disease, atherosclerosis, and Smith-Lemli-Opitz syndrome (SLOS) are associated with higher amount of cholesterol. On the other hand, it takes part in several important biological functions such as the synthesis of hormones and therefore limited CHO is necessary for human growth [1,2]. The main source of CHO in the human body is the intake of animal food such as eggs, meat, and dairy products, whereas it is also synthesized in the human body. Primary importance of cholesterol reduction is to prefer diet with low fat [1]. Due to the prominent physiological role of CHO its qualitative and quantitative determination in various foods is very important. Formerly, CHO and its metabolites were determined by various methods, such as thin layer chromatography (TLC), nuclear magnetic resonance (NMR), and enzymatic assay [2]. Liquid chromatography mass spectrometry (LC-MS), gas chromatography mass spectrometry (GC/MS), and calorimetric maximum extraction assays are, however, the most commonly accepted methods for precise and quantitative studies. CHO ionized with electron ionization (EI), therefore, it is a suitable candidate for GC/MS detection. CHO free form is present in animal fat after that adequate sample preparation, GC method could be used for direct determination [3]. In addition, liquid chromatography technique is also reported for the determination of cholesterol. However, this technique is not suitable for analyzing CHO molecule because of its low acidity and low proton affinity for electron spray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI) [4]. Chromatographic techniques require the use of large volumes of organic solvents and equipment that may not be present in many research laboratories [5], therefore alternative simple techniques are always needed. Spectrophotometry is a simple technique that can be used for the determination of a single

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compound after suitable sample cleanup. Cholesterol absorbs light in the lower UV region consequently; its determination in biological samples or food is very difficult due to possible interference by endogenous compounds or another sample matrix. Extraction of cholesterol with *n*-hexane also results in co-extraction of closely soluble compounds which hamper its spectrophotometric detection. To overcome this problem, extraction of solid phases has been performed. Thin layer chromatography (TLC) and solid phase extraction (SPE) are the most common and cheapest purification techniques. SPE is conducted on a variety of adsorbents, like activated carbon, silica gel, zeolites and ion exchange resins [6]. Depending on the matrix, the samples can be cleaned to get nearly pure compounds. However, to achieve the required specificity, selective binding sites are introduced in SPE materials. The most popular selective binding materials are molecularly imprinted polymers (MIPs), calixarene and crown ethers, *etc*. [7]. Selectivity of these materials is attributed to their cavities and specific covalent Metal Organic Frameworks (MOF) are relatively new class of hybrid inorganic/organic porous materials which possess cavities (porous in nature) and have specific binding sites similar to MIPs, calixarenes and crown ethers. In recent years, MOFs attracted much attention due to their large surface area, tunable pore structure, and high thermal stability [8]. MOFs are synthesized from secondary building units (SBUs) which are inorganic parts (either a single coordinated metal ion or an oligo-nuclear metal cluster) and linkers that must have a rigid geometry and can be functionalized to modify the properties of the framework. These new classes of porous materials have found applications in gas storage, catalysis, separation, adsorption, sensors, and drug delivery. The large variety of structures, pore size, high surface area, excellent adsorption affinity, and selective penetration have made MOFs attractive as sorbents [9]. MOFs are previously used for stir bar sorptive extraction, solid phase extraction and solid phase micro extraction [10]. MOFs are ultrafine crystalline materials when dissolved in liquid they are dispersed and require high quality of filtration to separate the MOFs loaded with the analyte. Therefore, immobilization of MOF onto the supports that can hold them without compromising the MOF selectivity or porosity could be a feasible option in the field of selective extraction for chemical analysis.

Cryogel has been reported as a successful immobilization medium for numerous molecules [11]. Cryogel is a super-macroporous hydrogel showing excellent flow-through properties. Therefore, the particles pass freely from the cryogel, hence it provides efficient mass transfer [12]. Thereby, cryogels are employed to embed different types of particles and adsorbents in its polymeric network and can be used as a robust composite matrix [13]. Selective incorporation of adsorbents embedded into cryogel leads to the porous structure, which is suitable for the separation of small/large target molecules. One-step polymerization either from monomer/polymer solutions or particle suspensions under cryotropic conditions can be used to prepare composite cryogels [14].

According to the aforementioned, this work deals with the immobilization of MOFs into cryogels, which makes it easier to handle them without any loss or leakage of the adsorbent, which would otherwise lead to lower efficiency after running several cycles. On the other hand, by embedding particles in a cryogel matrix, the adsorption/desorption operation can also be carried out in continuous mode, which shortens the operation time. In the current study, MOF-5 (Zn_4O clusters connected by 1,4-benzene dicarboxylate (BDC) linkers) was embedded in cryogel and applied to selectively extract cholesterol from milk samples followed by determination of cholesterol using simple UV-Vis spectrophotometric method. Herein, we have reported the preparation of the composite, its characterization, and various parameters that affect the selective extraction of cholesterol from milk samples.

2. Experimental

2.1. Materials

The chemicals such as 1,4-benzenedicarboxylate (BDC), zinc nitrate ($Zn(NO_3)_2{\cdot}6H_2O$), ethanol (C_2H_5OH), ethyl acetate ($CH_3COOC_2H_5$), carbon tetrachloride (CCl_4) and acetic acid were purchased from E. Merck, Darmstadt (Germany). Cholesterol was purchased from BDH Chemicals Ltd., Poole, (England). Methanol (CH_3OH) and *n*-hexane were purchased from Dae-Jung Chemicals & Metals Co., Ltd. The chemicals, dimethyl sulfoxide (DMSO) and potassium hydroxide (KOH) were purchased from Fisher Scientific, UK. *N*,*N*,*N*,*N*'-Tetramethyl ethylenediamine (TEMED) was purchased from USA and ammonium persulfate (APS) was purchased from Kuksan, (Korea). 2-Hydroxyethyl methacrylate (HEMA) was purchased from Alfa Aesar (England) and ethylene glycol dimethacrylate (EGDMA) was purchased from Kasei Kogyo Co., Ltd., (Tokyo). Dimethylformamide (DMF) was purchased from Tianjin Standard Chemical Reagent Co. Ltd. (Tianjin, China), and chloroform ($CHCl_3$) was purchased from Fisher Scientific, UK.

2.2. Instrumentation

The morphology of synthesized MOF-5 composite was investigated by scanning electron microscope (SEM) (JSM-6491 LV, Joel, Japan), Energy dispersive X-ray (EDX) analysis (JSM-6492 LV, Joel, Japan) and FTIR (Nicolet 5700 Thermo Electron Corporation, USA). Powder X-ray diffractometer (PXRD) XPERT-PRO (Goniometer PW3050/60) was used to determine the Braggs angle and the peak intensities. UV-Visible spectrophotometer (Cary-100, Agilent Technologies; USA) was operated at room temperature (200-800 nm) to optimize different parameters throughout the study.

2.3. Preparation of MOF-5 composite

MOF-5 particles were prepared by a solvothermal method described earlier [8]. The colourless crystals were collected; washed through DMF, and dried at ambient temperature. MOF-5 was immobilized into cryogel with free radical cryogelation process. MOF-5 100 mg was dispersed in a mixture of DMSO and deionized water (1:2, *v*:*v*, mL) for 30 min. Then, 0.65 mL of HEMA and 0.25 mL of EDGMA were mixed well and added to the MOF-5 solution mixture and then solution was cooled in a refrigerator but not allowed to freeze. Then, APS (185 µL from 10% stock solution, 1% *w*:*v*) and TEMED (18.5 µL, 1% *w*:*v*) were added in the above mixture maintained at 0 °C in an ice bath. Immediately, the reaction mixture was poured between two glass plates separated by 1.5 mm thick spacers. The polymerization solution between the plates was frozen at -16 °C for 24 h and then thawed at room temperature. The resulting cryogel sheet was cut into circular pieces (0.5 cm diameter) with a perforator. The MOF-5 embedded composite cryogel discs were washed several times with water to remove nonreacted monomers and to check the leaching of particles.

2.3.1. Optimization of parameters evaluation of uptake capacity

For the measurement of adsorption capacity; (1) a dried MOF-5/cryogel composite disc was added into the 5 mL solution of cholesterol with different concentrations ranging from 1 to 100 µg/mL in *n*-hexane. The resulting solutions were shaken for 3 h in an orbital shaker at 100 rpm at ambient temperature. After the completion of the adsorption process, MOF-5/cryogel composite disc was removed and the

unabsorbed concentration of cholesterol was checked via UV-Visible spectrophotometer. The triplicate data was obtained and mean±standard deviation (±SD) was calculated. The reason behind the MOF-5 embedded composite cryogel preparation is that when we use MOF-5 particles directly, some of them dissolved after shaking and the solution turns turbid not found good results.

2.3.2. Effect of loading amount of MOF-5 into cryogel

Diverse amounts of MOF-5 were embedded to check out the adsorption efficiency of MOF-5/cryogel composite for cholesterol. Cryogel were prepared by loading 50 to 200 mg of MOF-5 and adsorption was carried out using 60 µg/mL standard solution of cholesterol. Concentration of cholesterol after adsorption and respective standard solution was estimated by measuring the absorbance using UV-Vis spectrophotometer. Adsorption experiments were carried out using dried and wet MOF-5/cryogel composite discs and adsorption capacities were compared. 100 mg of MOF-5 loaded disc gives good % sorption, so we have selected 100 mg for further study.

2.3.3. Effect of contact time

To evaluate the optimum time for uptake of cholesterol on MOF-5/cryogel composite, a solution of cholesterol in *n*-hexane (5 mL of 60 µg/mL) was taken into seven different 25 mL glass flasks each containing 1 disc of MOF-5/cryogel composite. Solutions were shaken at a speed of 100 rpm for 30-210 minutes at ambient temperature with an interval of 30 minutes. Subsequently, filtered and analyzed via UV-Visible spectrophotometer. Mean±standard deviation (±SD) was calculated from the obtained results.

2.3.4. Effect of desorption solvent and desorption contact time

Various solvents; *n*-hexane, chloroform, ethyl acetate, carbon tetrachloride, and a mixture of three solvents $CHCl_3$: C_2H_5OH: CH_3COOH in the ratio (3:1:1, *v*:*v*:*v*) were tried for desorption. The optimum desorption contact time was monitored by taking 5 mL of desorption solvent and added into ten separate 25 mL glass flasks, which were then shaken (100 rpm) for 30-210 minutes at room temperature. Subsequently, the mixtures were filtered and analyzed. Precision and mean±standard deviation (±SD) were calculated from the obtained results.

2.4. Validation of the proposed method

The method was established through various quality parameters; sensitivity, precision, linearity, accuracy, and limits of detection and quantification. In the present study, MOF-5/cryogel composite was used for cleanup of cholesterol samples. Therefore, for the determination of quality parameters, adsorption of the analyte was followed by desorption and the recovered analyte was measured spectrophotometrically in triplicate analysis. The regression line was calculated by using least square method and expressed by the determination of coefficient (r^2) [15]. Limits of detection and quantification were calculated as concentrations corresponding to three times and ten times of standard deviation of blank samples.

Precision was calculated both as inter- and intra-day variation (n = 3). The method was further proposed for the determination of cholesterol in powder milk samples of five different brands; the accuracy of three liquid milk samples was checked through recovery studies and comparing the results with HPLC procedure [16].

2.5. SPE and UV-Vis determination of cholesterol using MOF-5/cryogel composite

Milk samples were collected from local markets of Hyderabad, Pakistan. Various batches of five different brands of powder milk samples were purchased from supermarkets and pooled to give a composite of each brand. Likewise, liquid milk samples were purchased from dairy and samples collected on each day were pooled to obtain one composite sample. Liquid milk samples were collected for three consecutive days from various locations of Hyderabad, Pakistan. Cholesterol was extracted from the milk in *n*-hexane by a method described previously [16]. Pretreatment of the sample is done through SPE, MOF-5 poses open metal sites, cholesterol selected is an analyte due to the hydrophobic and acid-base interaction occurs in between them, hydroxyl groups of cholesterol participate in hydrogen bonding [17]. Then, approximately 0.015 g of cryogel composite disc was accurately weighed and placed in a 25 mL glass flask. 5 mL of standard cholesterol solution (*n*-hexane) was added to it; subsequently, the flask was placed by shaking for 4 h until the maximum amount of cholesterol was adsorbed on cryogel composite. After adsorption, the cryogel composite disc was taken out and dried. Then, the cryogel composite disc containing cholesterol was added in 5 mL desorption solvent which is made up of a mixture of solvents ($CHCl_3$: C_2H_5OH: CH_3COOH) in ratio 3:1:1 (*v*:*v*:*v*). Flask was placed for shaking up to 4 h for maximum desorption of cholesterol. Filtered samples were run on UV/Vis spectrophotometer and data was obtained in triplicate and reported as mean±standard deviation (±SD).

3. Results and discussion

Prepared material (MOF-5) was white crystals whereas its composite was chunky and foamy white material. The total yield of the material was 1.0 g including 100 mg of MOF-5 in the composite, which means composite material contains 10% MOF-5 in its structure. Furthermore, MOF-5 and newly prepared materials were characterized for composition and morphology using FTIR, Powder-XRD and SEM-EDX imaging.

3.1. Characterization of MOF-5 composite

Powder X-ray Diffraction Patterns of the synthesized MOF-5, MOF-5/cryogel composite, and cryogel MOF-5 showed a prominent diffraction peak at a 2-theta value of 6.78° and a few other less intense diffraction peaks (Figure 1). The PXRD pattern is similar to IRMOF-1 (MOF-5) as reported by Yaghi *et al*. using the close vessel method [17] and compared with [18]. which confirms the formation of MOF-5 under hydrothermal conditions.

FTIR spectra of MOF-5 closely resembled to its microcrystalline state as described by Hafizovic *et al.* [19]. There was no peak around 1700 cm^{-1} which shows that all carboxylates of BDC are in deprotonated form and bound to Zn atom. Characteristics peaks of MOF-5 were observed as; CH stretching vibration at 3150 cm^{-1}, asymmetric stretch of O_2C-C_6H_4 at 1550 cm^{-1}, CC ring stretch at 1370 cm^{-1}, asymmetric OCO bending at 800 cm^{-1} and out-of-plane COO^- CH bending at 748 cm^{-1} as reported by reference [20]. MOF-5/Cryogel composite vibration frequencies showed close match with a report on poly (HEMA) co-EGDMA by Perez-Salinas *et al.* [21]. Wavenumber of 3420 cm^{-1} for OH from HEMA, 2990 cm^{-1} for CH from EGDMA and HEMA, 1717 cm^{-1} for C=O from EGDMA and HEMA structures, 1450 cm^{-1} from CH from EGDMA and HEMA, 1320 to 1300 cm^{-1} of C-O- ester from HEMA and EGDMA, 1170 cm^{-1} for C-O- carboxylic derivate confirms the presence of poly (HEMA) co-EGDMA on surface of composite.

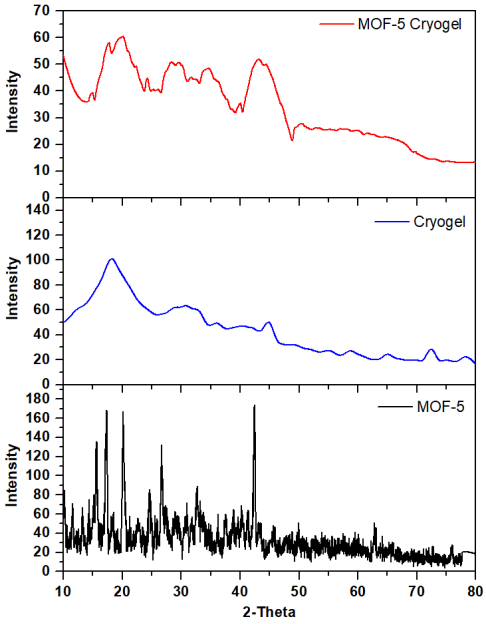


Figure 1. Powder X-ray diffraction patterns of the synthesized MOF-5, cryogel and MOF-5/cryogel composites. Powder X-ray diffraction pattern of MOF-5: Reference PXRD pattern from [18].

In simple cryogel, an intense peak was appeared at 1200 cm^{-1} of C-O- carboxylic group in poly (HEMA) and EGDMA. In addition, the peak at 1600 cm^{-1} regarding the stretching vibration of carbonyl group belongs to the amide bond. The characteristic sharp peak at 1690 cm^{-1} confirms the stretching vibration band of C=O group of HEMA and EGDMA. Moreover, the peaks ascribed to free monomers by Perez-Salinas *et al.* [21] were not found in composite showing the gel does not contain any monomers. The composite material does not show any bands attributed to MOF-5, but it is dominated with peaks from poly (HEMA) co-EGDMA which suggest that most of the surface of the composite material is covered with cryogel or MOF-5 is not accessible in attenuated total reflectance (ATR) mode of FTIR.

Furthermore, the morphology and compositing effect was investigated through SEM and EDX imaging. Figure 2 shows the SEM image of MOF-5 which reveals that most of the particles are heterogeneous in size, the particle size varies from 10-100 µm, and the morphology of the particles is cube-shaped which supports the data obtained from PXRD that crystals are microcrystalline. The same figure shows SEM images of cryogel without MOF-5 particles and MOF-5 embedded cryogel composite as well. Two images look much similar showing that embedding of MOF-5 into cryogel does not alter the morphological structure of cryogel at the microscopic level. Surface roughness which results from the porosity of cryogel is obvious in both images. Blending of MOF-5 with cryogel was confirmed by taking an elemental mapping image through EDX (Figure 2) (three images at the bottom in colour) which reveals that Zn (presents MOF-5) is blended with cryogel and is highlighted with green patches.

3.2. Optimization of solid phase extraction parameters

SPE is a two-step process where the analyte is taken up from solution on the surface of the solid material which is then removed using desorption solvent. For accurate quantification of an analyte, the uptake capacity and then quantitative desorption of analyte should be optimized. To achieve such conditions, new materials for SPE need to be optimized for various parameters that may affect the adsorption/desorption process. The most significant parameters involving the uptake of cholesterol from *n*-hexane solution by the MOF-5/cryogel composite are; contact time, composite/sorbent amount, desorption solvent and desorption contact time. Here, the physical state of the composite (wet/dry) was also investigated along with above mentioned parameters.

As synthesized frozen MOF-5/cryogel composites which contain water in its structure and room temperature dried composite were studied for adsorption of cholesterol from *n*-hexane solution. Dried cryogel showed very good adsorption of cholesterol up to 70% as compared to 45% sorption on the wet gel. Therefore, all other studies were performed with dried composites. This may be due to the better transport of *n*-hexane into the dried gel as compared to wet gels which contain water in its structure. Figure 3a indicates the uptake capacity of cholesterol onto MOF-5/cryogel composite which was increased along with increased initial concentration till the concentration reached to 60 µg/mL. Uptake capacity curve at 4 h contact time became relatively flat and reached saturation at high concentrations. The effect of uptake time was investigated (0.5 to 6 h), at intervals of 30 min, Figure 3b. It was found that the uptake capacity was increased with increasing contact time. It reveals that after 4 h the maximum uptake capacity 13.04 (mg/g) was found and corresponded to 85% uptake of cholesterol from *n*-hexane solution. Longer contact of equilibrium of cholesterol and MOF-5/cryogel composite shows that the diffusion of cholesterol is slow which may be due to its preferential solubility in *n*-hexane and adsorption on microporous MOF-5. A similar finding for slow kinetics of adsorption is reported for the uptake of oxybenzene onto metal-organic frameworks. Maximum uptake was achieved after 15 h contact time [22].

Similar experiments were also performed with cryogel composites of MOF-5 which show low uptake of cholesterol from *n*-hexane solution; the selectivity of MOF-5 towards cholesterol may be attributed to dimensions of the cubic cages present in MOF-5, and thus the corresponding void volumes. It is also reported that MOF-5 showed good sorption for non-polar polycyclic hydrocarbons, but polar molecules showed lower or no affinity for MOF-5 [23].

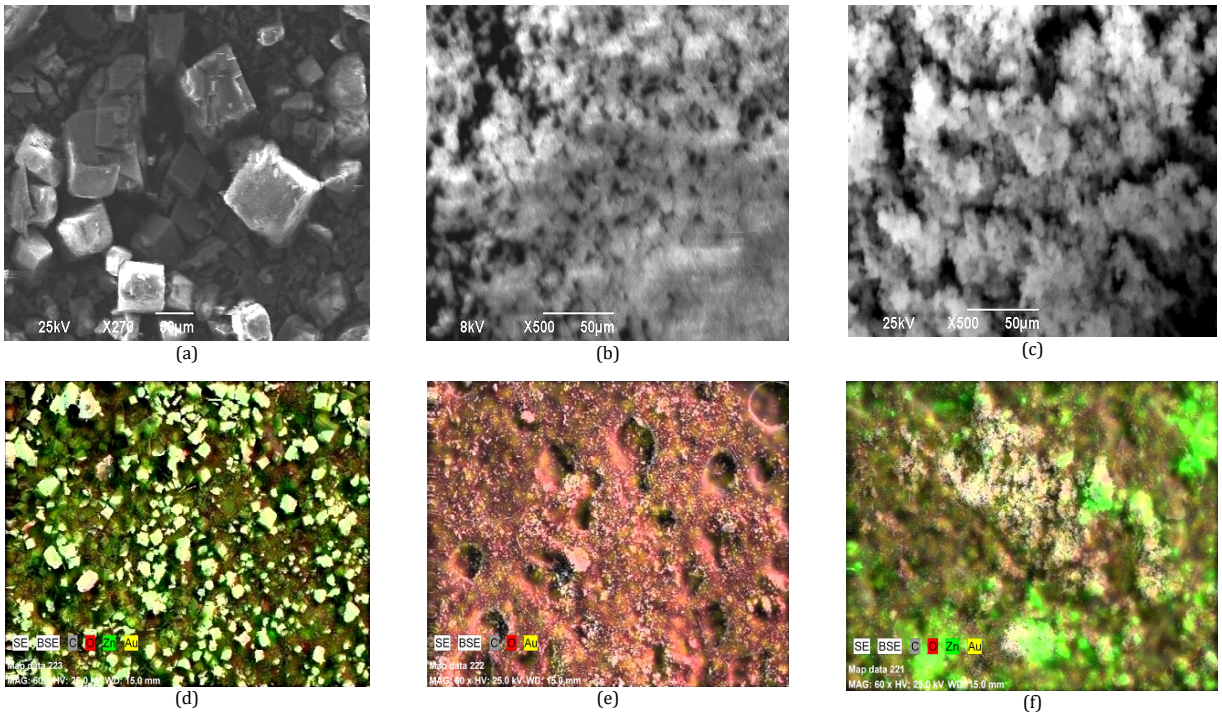


Figure 2. SEM image of MOF-5 (a); SEM image of cryogel (b); SEM image of MOF-5 embedded cryogel composite (c). EDX mapping of the same sample area, demonstrating the presence of oxygen, carbon and zinc: MOF-5 (d); Cryogel (e); MOF-5 embedded cryogel composite (f).

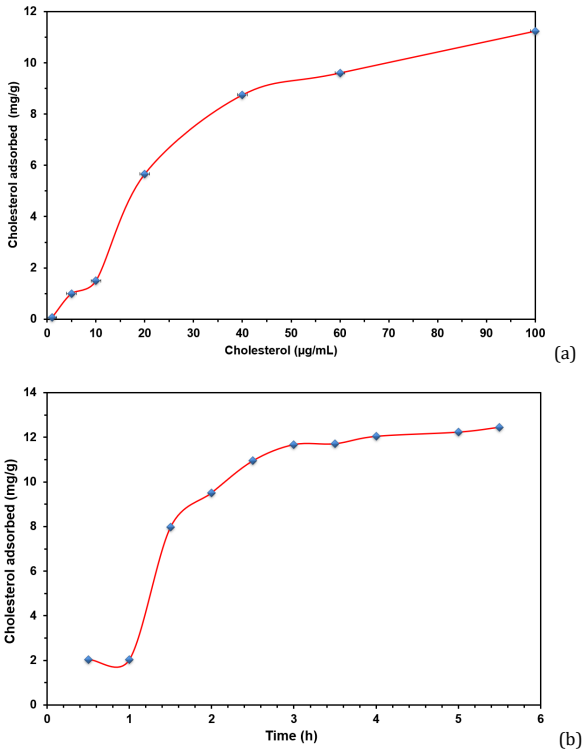


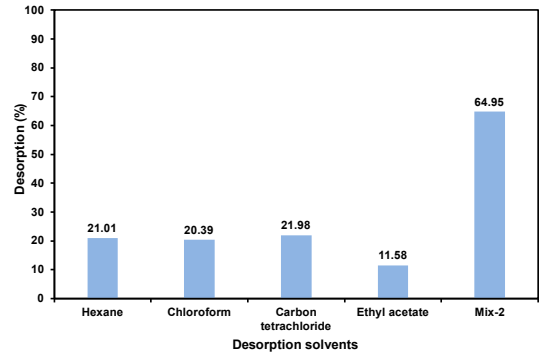
Figure 3. (a) Effect of cholesterol concentration on its uptake by MOF-5 composite; Conditions: Solution volume (5 mL), contact time (4 h), amount of composite (100 mg). (b). Effect of contact time; Conditions: Cholesterol concentration (60 µg/mL), solution volume (5 mL), amount of composite (100 mg).

To verify the role of MOF-5, experiments were performed with simple cryogel without embedding MOF-5, cryogel gives lower percent sorption (38%) compared to MOF-5/cryogel composite, which gives (59%) sorption. Therefore, it may be suggested that the selective nature of MOF-5 towards nonpolar cyclic hydrocarbons is responsible for the uptake of cholesterol.

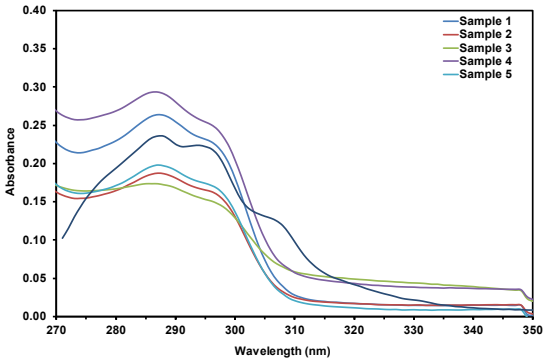
Selection of an appropriate desorption solvent is of crucial importance for a material to be used as an absorbent in solid phase extraction for sample clean-up. Therefore, neat solvents like chloroform, *n*-hexane, carbon tetrachloride, ethyl acetate, and various mixtures; Mix-1 ($CHCl_3$:CH_3OH in 2:1, *v*:*v*), Mix-2 ($CHCl_3$: C_2H_5OH: CH_3COOH in the ratio of 3:1:1, *v*:*v*:*v*) and Mix-3

Table 1. Amount of cholesterol in different milk samples using the UV/Vis method.

Powder milk samples (100 mg/100 g)	Newly developed method (mg/g)	Labeled amount (mg/g)
S1	62.97	50
S2	52.59	65
S3	40.40	30
S4	37.74	40
S5	39.50	35
Liquid milk samples (100 mg/100 mL)		
S1	1.03	NA
S2	1.45	NA
S3	8.84	NA



(a)



(b)

Figure 4. (a) Effect of desorption solvent, Conditions: Solution volume (5 mL), amount of composite (100 mg). (b) Various milk samples after clean-up and standard in solvent Mix-2. Standard is highlighted with markers for clarity.

(CCl_4: *n*-hexane, 1:1, *v*:*v*) were studied as desorption solvents. Figure 4a shows desorption of cholesterol into various solvents where Mix-2 showed a maximum recovery of nearly 80% at 4 h contact time.

After studying the uptake of cholesterol from *n*-hexane solution, the composite material was employed for clean-up of milk samples containing cholesterol, Figure 4b. UV-Vis spectra of the cleaned samples are very close to the pure cholesterol standard which indicates the good efficiency of the composite to be used as solid phase extraction material. Henceforth, the method validation parameters were studied to build the authenticity of analytical protocol.

3.3. Validation of MOF-5/cryogel composite method for cholesterol

Composite of MOF-5/cryogel is easy to prepare and does not require multiple steps before use. Therefore, the optimized conditions for cholesterol detection and extraction in the course of SPE are straight forward and feasible. Cholesterol in milk samples was determined by the developed method using UV/Vis as the determination technique followed by SPE. Cholesterol adsorption percentage on the MOF-5/cryogel composite in the sample loading was 84% and cholesterol desorptions were 80% in the samples of milk. Therefore, a conversion factor was used to calculate final results in real samples. Cholesterol standard solutions in *n*-hexane were used for quantification by using an external calibration curve which was found in the range of 1-50 µg/mL with a coefficient of determination r^2 = 0.990. Detection limit (0.15 µg/mL) and quantification (0.45 µg/mL) were calculated. MOF-5 composite-based SPE material was also used to calculate the intraday precision of cholesterol concentrations at various concentration levels (1, 50, and 100 µg/mL) for n = 5 for each; 1.26, 1.31 and 1.67% relative standard deviation (RSD) were obtained. In addition, five measurements were calculated for the same concentration of cholesterol for interday precision n = 5 (three successive effective days), RSD values of 1.34, 1.37, and 2.29 % were observed. Developed method was applied for the analysis of different powder milk samples, in order to check the labeled amount is present or not the results were compared with HPLC in Table 1 and Figure 4b. In addition, very good recoveries (85 to 90 %) were obtained when the identified cholesterol amount was added to milk samples; the results showed good accuracy.

Table 1 shows that the labelled amount of cholesterol for brand S1 is 62.97 mg / 100 mL whereas the value obtained through HPLC method is (67.00 mg/ mL). Other samples also show variation in the labelled amount when found through the developed method. To ensure the results obtained, results were compared with HPLC-UV, and it revealed that the new method is good for the determination of cholesterol in milk samples. Furthermore, it may be observed from the data that most of the labeled amount in powder milk samples is different than the

labeled amount but within the average of 100 mg/100 g for powder milk samples reported by USDA database. Likewise, milk samples were also within the range of 10 mg/100 mL.

4. Conclusions

Newly prepared cryogels were found capable of holding MOF-5 microcrystalline material and resulting composites which can be used for hosting small molecules like cholesterol. The composite material was found good as solid phase extraction medium for sample clean-up of complex matrices like liquid and powdered milk. Sample cleaning was sufficient to reliably analyze cholesterol using simple spectrophotometer in UV range. The composite material also shows the potential for removing cholesterol, therefore after biocompatibility test, it can be used to produce low cholesterol milk for patients with hypercholesterolemia.

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Disclosure statement

Conflict of interests: The authors declare that they have no conflict of interest.
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