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Diethylaminoethyl cellulose (DEAE-C): applications in chromatography and organic synthesis

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Dedicated to the memory of Prof. Paolo Grünanger

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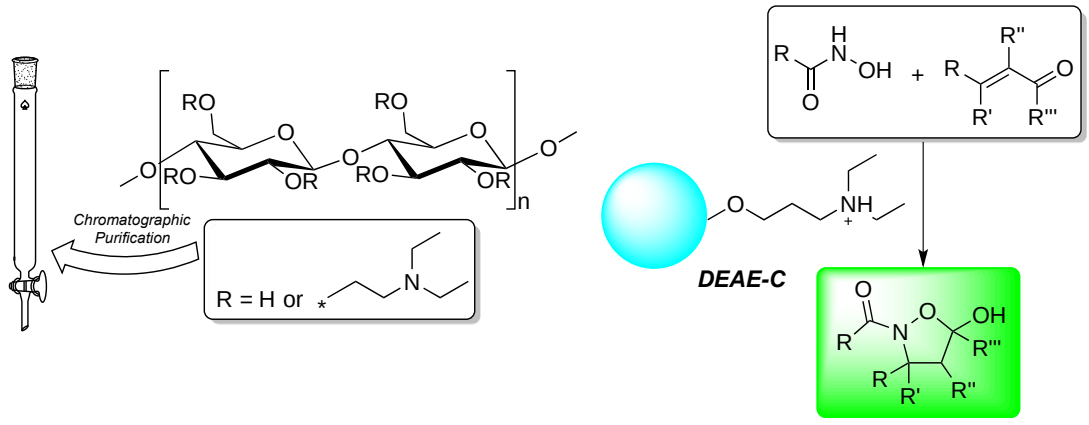
Abstract

The aim of this review is to point out the attention of the reader to the use of DEAE-C in organic reactions, possibly not only devoted to the preparation of heterocycles but potentially extending to other classes of organic compounds. Being DEAE-C an ammonium salt commonly used in chromatographic applications, it can be considered as a potential mild acid catalyst or a proton donor and these features can in theory catalyze standard acid-catalyzed organic reactions. In addition, the resin nature of DEAE-C could suggest the way to perform organic reactions in the solid state.



Keywords: DEAE-C, chromatography, organic synthesis, isoxazolidine

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1. Introduction

Diethylaminoethyl Cellulose (DEAE-C) is a positively charged resin typically used in ion-exchange chromatography for the separation of biomolecules and specifically the purification of proteins and nucleic acids. DEAE-C is a weakly basic ion exchanger with tertiary amine functional groups bound to a hydrophilic matrix (Figure 1).[1]

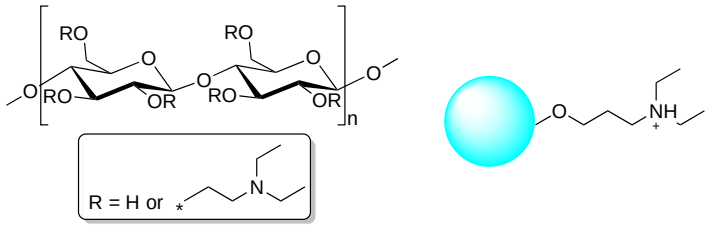


Figure 1. Chemical structure of DEAE-C.

DEAE-C is a commercially available compound with different names depending on the chemical suppliers (DE52 or DE53). They are prepared as pre-swollen material although cellulose exchangers swell in a strongly basic environment to increase access to binding sites. DEAE-C (*e.g.* DE52) has a pKa of 11.5 and the buffering range for diethanolamine is 8.4-8.8, though the range for DEAE-C varies between manufacturers.

DEAE-C is widely used as anion exchanger and the addition of the DEAE branch to the cellulose backbone also increases its ability to chelate metals in aqueous solutions, paving the way for its use as a ligand. The effectiveness of DEAE-C to perform these functions is perceived by its degree of substitution (DS). A method to increase the DS of the DEAE-C is to functionalize the cellulose in a homogeneous solution. Historically, this methodology was quite difficult due to the harsh solvents required to dissolve cellulose. Looking for possible alternatives, ionic liquids were found to offer a green solution to this problem, as they can be used as alternatives to traditionally harsh biopolymer solvents. Reichert and co-workers focused on the modification of cellulose into DEAE-C using a homogeneous ionic liquid with two functionalization procedures.[2]

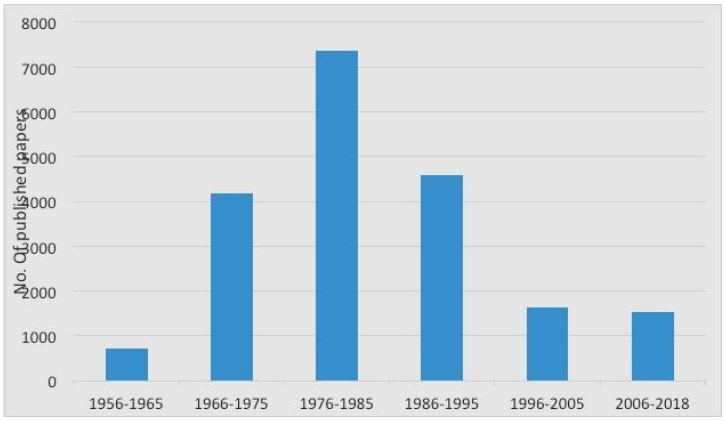


Figure 2. Number of published articles on DEAE-C over the period of years.

Regarding the applications of DEAE-C in organic chemistry, the number of published articles on this functionalized cellulose from 1956 to 2018 has increased remarkably up to the mid eighties with the publication of more than 7000 papers. In the next years, there was a rapid decrease in the number of papers dealing with this topic, reaching a stationary number up to nowadays (less than 2000 papers), as shown in Figure 2. In the year 2019 only 24 papers were published regarding DEAE-C.

The fields of application of DEAE-C are remarkably diverse; the main reasons for this wide application include the availability of commercial DEAE-C, even though only in recent years a few applications in organic chemistry can be found. In the majority of the cases, DEAE-C was used as an adsorbent. In this review we wish to illustrate the main, most relevant and recent applications in some chemical fields, finally focusing attention on the use in organic synthesis as an unconventional catalyst, with potential perspectives.

2. Purification of Proteins

One of the main DEAE-C application fields, as stationary phase in chromatography, is in protein purification. As known, proteins, the building block of life, play a pivotal role in all the aspects of the cellular system and their purification is a basic necessity. They are involved in almost all the cellular functions; from structural stabilization to the transport of nutrients, ions, and small molecules from immune defense to enzymatic activities.[3] Ion exchange chromatography (IEC) has been in common use for more than 50 years for the separation and purification of proteins. As a consequence, a great deal of literature documenting the widespread experience with IEC testifies the importance of one of the most utilized methods of protein separation techniques. Up to the year 1997, in 134 purification protocols 426 chromatographic steps were used and IEC constituted the 40%, gel filtration the 18%, and affinity chromatography the 29%, including immobilized metal affinity and dye affinity chromatography.[4] However, in the year range 2000-2005, the use of IEC is in decline and accounts only for 14% of chromatographic methods, and affinity (AC) and size exclusion (SEC) chromatographic methods are the most utilized methods.[4]

To cite some examples where DEAE-C is employed, Mayhew and Howell reported the results concerning two chromatographic techniques, where the procedures include the salting-out by high concentrations of ammonium sulfate and ion-exchange chromatography on a column of DEAE-C.[5] In IEC procedures, it is often necessary to remove excess salt before application of the protein mixture to the column. This is done to ensure that the ionic strength of the solution is sufficiently low for adsorption of at

least some of the proteins in the mixture. Lovenberg and Williams noticed that rubredoxin and ferredoxin, bacterial iron-proteins, are preserved by a DEAE-C column in the presence of a solution with high concentration of ammonium sulfate.[5] Both proteins are eluted and efficiently separated by different concentrations of salt.

In 2018 Soukhtehzari[6] reported that the Nuclear Mitotic Apparatus protein (NuMA) plays a critical role in the mitosis as an organizer of the mitotic spindles; this is a prevalent protein in malignant urothelial cells. Hence, NuMA is an appropriate marker for bladder cancer early detection. On the basis of literature studies, NuMA protein sequence was obtained from Genbank. The expressed protein was examined by SDS-PAGE to confirm expression by using anti-His antibody-HRP. The protein was then purified using nickel chromatography column and 18 mg of purified truncated NuMA were obtained from 1 L of bacterial culture. ELISA was performed to evaluate the reactivity of commercial anti-NuMA antibody towards truncated protein. The immunoglobulins of antiserum were precipitated by ammonium sulfate and purified by DEAE-C chromatography column. To evaluate antibody accuracy, it was tested on HEp-2 cells with permeable nucleus membranes fixed on slides. The antibody detected NuMA protein in the nuclei of HEp-2 cells; this means we can have diagnostic values in the bladder cancer patients. The truncated NuMA is a suitable protein as a single multiepitope antigen that further may be used for studies to develop assays for early diagnosis of bladder cancer.

Gayatridevi *et al.*[7] reported that storage proteins, such as arylophorin, are synthesized from the fat body of a wide range of lepidopteran and dipteran larvae and in other insect orders. Storage protein from hemolymph of *H. armigera* was obtained by ammonium sulphate fractionation and anion-exchange chromatography. From 1,500 mg of total protein, 7.35 mg of purified protein was acquired with 24% recovery. The native molecular weight of the protein was determined to be 400 kDa. Multiple amino acid sequence alignment of obtained LC-MS sequence of the purified protein of *H. armigera* indicated that the protein is arylophorin. The deduced amino acid sequence was found to more homology with the arylphorins of *Spodoptera litura, Manduca sexta, Hyalophora cecropia* and *Antherae apernyi.* Hemolymph from the late instars served as the starting material for the purification of storage proteins. An indispensable first step was fractionation by ammonium sulphate. At first, the dialyzed hemolymph was saturated with 40%; later the precipitation rose up to 60%. The precipitated proteins were subjected to anion exchange chromatography. The storage protein was eluted as a single peak from DEAE-C, and gave a single band when analyzed by SDS-PAGE.

These few examples have been reported here to show the very first use of DEAE-C in a remarkably important field such as that of protein purification. Literature in this field also reports a huge number of studies where the pH can manipulate the charged character of amino acids and proteins, in particular. There is no need to emphasize how the obtaining of purer and purer proteins continues to be a key point in biological investigations. However, this topic and the relationship with DEAE-C structure that contains a quaternary ammonium group has not been reported here since it relates to a specific interest in chemical biological investigations that goes beyond the scope of this review.

3. Polysaccharide Purification

DEAE-C finds another field of application in the purification of polysaccharides and here we report some relevant and recent examples in this topic.

Acanthophora muscoides (Rhodophyta) has three polysulfated fractions (Am-1, Am-2 and Am-3). Am-2 displayed anti-inflammation and serpin-independent anticoagulation effects. In the search of other biological applications it was found that these oligomers had no effects on thrombin-generation (TG). Hence, it was employed a mild-acid hydrolysis to obtain low-molecular-size derivatives from Am-2 and *in vitro* inhibitory tests were performed to compare the activities between intact Am-2 and its hydrolysates on a TG assay.

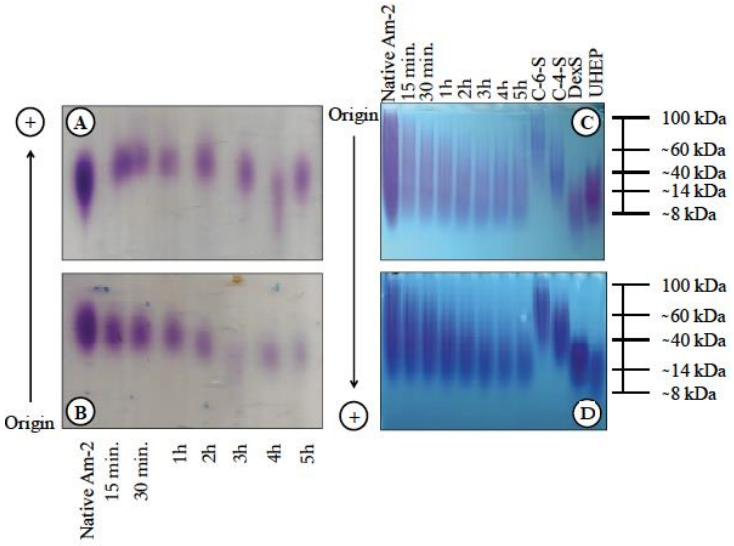


Figure 3. Electrophoresis in agarose gel (A and B) and in polyacrylamide gel (C and D) after the depolymerization procedure with 0.02 (A and C) or 0.04 (B and D) M HCl of Am-2, obtained by DEAE-C, from the red seaweed *A. muscoides*. Low molecular weight dextran sulfate (8 kDa, DexS), unfractionated HEP (14 kDa, UHEP), chondroitin-4-sulfate (40 kDa, C-4-S) and chondroitin-6-sulfate (60 kDa, C-6-S) were used as standards. SPs present on gels were stained with 0.1% toluidine blue.[8]

DEAE-C was used to fraction the polysaccharidic extract; Am-2 eluted with 0.75-M NaCl contained sulfate (23%), hexoses (51%) and absence of proteins, indicating structure of galactan similar to that of the extract obtained by one-dimension nuclear magnetic resonance. Regarding the TG assay, intact Am-2 inhibited concentration-dependent intrinsic pathway, whereas its hydrolysates abolished it like unfractionated heparin when in 60-fold diluted human plasma using chromogenic method (Figure 3). This can be an alternative approach for the production of oligosaccharides from *A. muscoides* with TG inhibition.[8,9]

Another example is that of Exopolysaccharide of Lachnum 7YM130 (LEP) (Figure 4) was purified by DEAE-C and Sepharose CL-6B column chromatography. LEP-2a was identified to be a homogeneous component with an average molecular weight of 1.31×10^6 Da, which was constituted by mannose and galactose in a molar ratio of 3.8:1.0.[10]

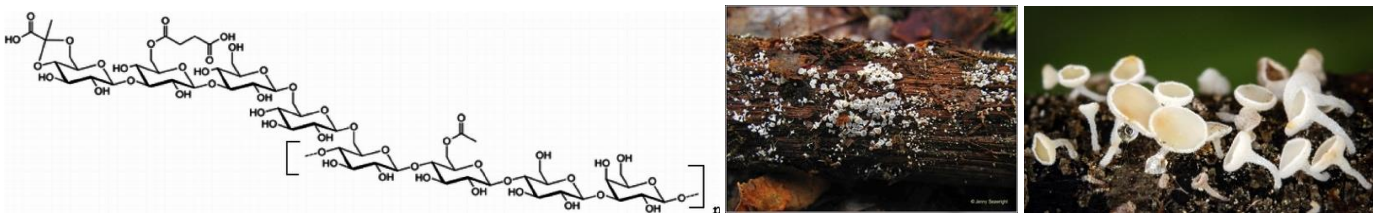
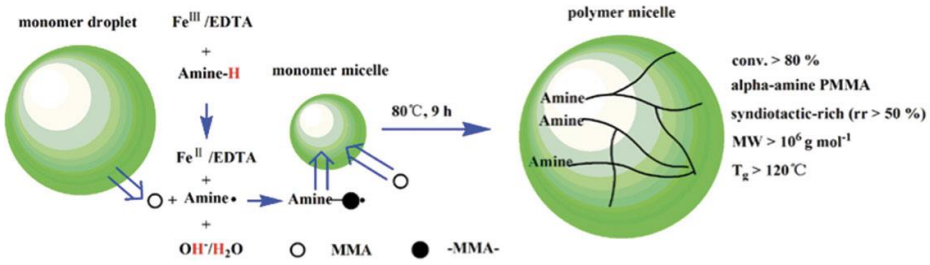


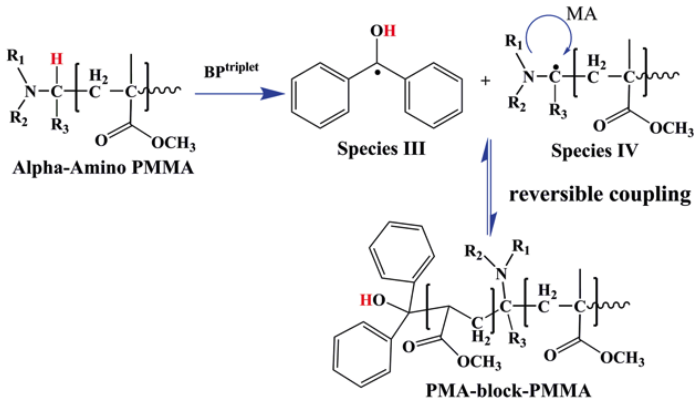
Figure 4. Structure of exopolysaccharide (pictures of Lachnum).

Quite remarkably, DEAE-C finds application in the following example. Some transition metal complexes in high oxidation state can oxidize tertiary amines under proper conditions into amino-alkyl radicals, able to initiate polymerization of electron-deficient vinylic monomers. They form mono-centered redox-initiation pairs suitable for the preparation of 100% alpha-amino telechelic polymer. Radical polymerization of methyl methacrylate (MMA) can be performed by using water-soluble amines and $FeCl_3$ or $CuSO_4.5H_2O$ as an oxidizing agent. Tertiary amines such as 2-(*N,N*-dialkylamino)ethanol and *N,N,N',N'*-tetramethylethylene-diamine exhibit a higher initiation activity. Monomer conversion can reach 80% in 8 h and 95% in 16 h, leading to PMMA with an absolute average molecular weight above 1.5 x 10^6 g mol^{-1} (Scheme 1).



Scheme 1. Radical polymerization of methyl methacrylate (MMA).[11]

Scheme 2 shows the UV-induced BP-initiated radical polymerization of MA using the alpha-amino poly methylmethacrylate (PMMA) as the macrosensitizer to prepare PMA-block-PMMA.[11]



Scheme 2. UV-induced BP-initiated radical polymerization of MA using the alpha-amino poly methylmethacrylate (PMMA).[11]

Another interesting example of DEAE-C application in purification technologies was illustrated by Xu and co-workers[12] who were involved into their studies on the root of *Pueraria lobata*, considered to be a medicinal and edible herb for the treatment of diabetes, having a long history of application in China. To explore the constituents responsible for the antihyperglycemic activities of *Pueraria lobata*, a water-soluble polysaccharide (Figure 5, PL70-1-1) was isolated and purified by using a DEAE-C 52 anion exchange column and a Sephacryl S-100 gel filtration column.

High performance gel permeation chromatography (HPGPC) was used to determine its molecular weight that was found to be 2584 Da. Its structure was deduced by Fourier transform-infrared spectroscopy (FT-IR); monosaccharide composition analysis was given on the basis of gas chromatography coupled with mass spectrometry (GC-MS) as well as upon nuclear magnetic resonance spectroscopy (NMR). It was deduced

that PL70-1-1 was a glucan, and its main chain consisted of (1→)-linked β-D-glucose, (1→4)-linked α-D-glucose, (1→4, 6)-linked β-D-glucose, and (1→3)- linked α-D-glucose, and the branch chain consisted of (1→)-linked β-D-glucose. PL70-1-1 did not have a triple-helix structure. Furthermore, PL70 and PL70-1 displayed selective inhibitory effects on α-amylase and α-glucosidase *in vitro*. PL70 had remarkable α-glucosidase inhibitory activity. However, PL70-1-1 exhibited outstanding α-amylase inhibitory activity, with an IC50 of 3.945 μM in vitro. This indicated that its activity was 417 times higher than the positive control acarbose. PL70-1-1 may be beneficial as an α-amylase inhibitor, reducing the postprandial blood glucose level and treating type 2 diabetes. In order to give a general idea of the wide applications of DEAE-C in the separation and purification of polysaccharides from natural sources, hereby we report in Table 1 a variety of examples where DEAE-C gave the best results.

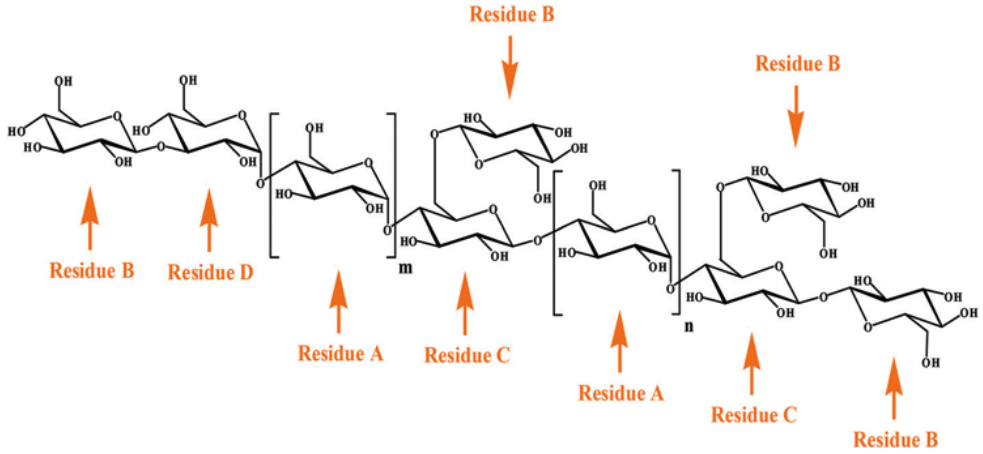


Figure 5. Structure of Polysaccharides of type PL70-1-1.[12]

In all the cases DEAE-C played a crucial role in separating the reported polysaccharides that were found to be biologically active, for example against cancer. Just to give few examples, the golden needle mushroom (*Flammulina velutipes*), a popular edible fungus in Taiwan and Asia, were used to isolate the flammutoxin that plays a crucial role in the immunomodulatory response.[13-15] Pectic compounds (pectic polysaccharides) form a group of complex and variable natural compounds. The term "pectic" means the group of oligosaccharides and polysaccharides, which have similar properties, but tremendous diverse fine structure. Pectic polysaccharides contain in fact the following structural elements: homogalacturonan (HG), rhamnogalacturonan-I (RGI), rhamnogalacturonan-II (RGII), apiogalacturonan (AGA), xylogalacturonan (XGA), arabinan, galactan, arabinoga lactam. An inhibition effect on A β42 aggregation and promotion of neuritogenesis is displayed by the pectic polysaccharide isolated from the *Lonicera japonica*.[16]

Polysaccharide HALPs1-1 is a glucan with an average molecular weight of 151.70 kDa, and HALPs2-1 was composed of glucuronic acid with an average molecular weight of 114.81 kDa.[17] AAPS-1 is a neutral polysaccharide, with a molecular weight of 23.2 kDa and comprised of Glucose, Galactose and Arabinose with a relative molar ratio of 1.6:5.1:1.0.[18] Polysaccharide SLAMP-a and two acidic polysaccharides (SLAMP-c and SLAMP-d) were obtained from stems and leaves of *A. Manihot* by DEAE-C chromatography. SLAMP-a is a water-insoluble mixture while its sulfated derivative (S-SLAMP-a3), prepared with aminosulfonic acid, is a homogeneous polysaccharide with excellent solubility. SLAMP-a and its sulfate mainly contained glucose, and SLAMP-c and SLAMP-d were both composed of mannose, rhamnose, glucuronic acid, glucose, galactose, and arabinose. *In vitro* study indicated that S-SLAMP-a3, SLAMP-c and SLAMP-d exhibited significant

immunomodulatory activity, while SLAMP-a showed little effects.[19,20] PIP2-1 is composed of mannose, glucose, galactose, fucose.[22] NFP-1 has a relative molecular weight of 950 kDa and mainly consists of galactose, arabinose, rhamnose, and galacturonic acid.[27]

Table 1. Polysaccharide extracts, natural sources and biological activities from DEAE-C separation and purification processes

Extract	Natural Source	Activity	Ref.
Flammutoxin	Flammulina velutipes	Immunomodulatory	13-15
Pectic polysaccharide	Lonicera japonica	A β42 Aggregation inhibit.; neuritogenesis	16
$HALP_{S1}$-1	Helicteres angustifolia	Antioxidant	17
Water-soluble polysaccharide	Acanthophyllum acerosum roots	Antioxidant	18
Chitinase	Aspergillus griseoaurantiacus[a]	Antifungal	19
SLAMP	Abelmoschus manihot[b]	Immunomodulatory activity	20
PRG1-1	Russula griseocarno	Antitumor activities	21
PIP2-1	Paxillus involutus	Antioxidant and immunomodulatory	22
RG1-Pectin	Bee pollen of Nelumbo nucifera	Immunological activity	23
Polysaccharide TFPB	Flower buds of Tussilago farfara	Proliferation inhibition of A549 cells and apoptosis.	24
Fucoidan	Sargassum henslowianum	Immunomodulatory effect on gastric cancer rat	25, 26
NFP-1	Nervilia fordii	Immunomodulating	27
PEP	Pachyrrhizus erosus	Anti-diabetic	28
Fucoidan	Sargassum polycystum	Antioxidant and anticancer	29
ESPS-1	Eremurus stenophyllus roots	Immunological	30
Pectic polysaccharide WOP-2	Okra (Malvacee)	Food ingredient, and traditional medicine in China	31

[a] KX010988. [b] Stems and leaves.

Polysaccharides were extracted from *Pachyrrhizus erosus* (PEP) and three fractions (PEP60, PEP80 and PEP95. The average molecular weight of PEP95-DS was 11.4 kDa, and it was composed of mannose, rhamnose, glucosamine, glucose, galactose, xylose, arabinose.[28] These latter extracts from different natural sources were found to be active as antioxidants, antifungal, antidiabetics or as immunomodulators.[27,28]

Russula griseocarno, flower buds of *Tussilago farfara*, Sargassum species gave extracts able to contrast cancer.[25,26,29] PRG1-1 has a molecular weight of 630 kDa and is composed of glucose, galactose, mannose, xylose and fructose.[21] TFPB1 was a homogeneous polysaccharide with a molecular weight of 37.8 kDa and composed of rhamnose, galacturonic acid, glucose, galactose, and arabinose, in a ratio of 13:13:1:7:12.[24] ESPS-1 is a 2-*O*-acetylgalactan, composed of galactose, arabinose and mannose in a molar ratio of about 10:3:1, respectively.

In summary, the DEAE-C based separation methodology revealed its efficiency in allowing for the isolation of biologically active compounds that find application in several fields, such as the isolation and purification of polysaccharides from a variety of different natural sources.[30,31]

4. Enzymes Purification

Enzymes are the reaction catalysts of biological systems, which accelerate and direct specific biochemical reactions. In order to illustrate remarkable examples of DEAE-C as purification system and trying to make a selection within the wide family of enzymes, let us focus the attention on antioxidant enzymes. These enzymes represent a category capable of stabilizing, or deactivating free radicals before they attack cellular components. Catalase (CAT) promotes the catalytic reduction of hydroperoxides, protecting mammalian cells against oxidative damage. The aim of an Indian research group was to analyze the presence of antioxidant enzyme and to purify the enzyme from the aquatic fern *Azolla* (Figure 6).

Figure 6. Aquatic fern *Azolla*.

First, the CAT enzyme presence was confirmed by standard assay procedure and, second, purified through DEAE cellulose and Sephadex G-75 Column chromatography. The purified CAT enzyme was subjected for molecular weight determination by SDS-PAGE analysis. Since the separated enzyme appeared as a single band, it was concluded that CAT enzyme was tetrameric. Maximum enzyme activity observed at pH 7 which was the optimum pH level of the CAT purified from *Azolla* and the optimum temperature level was 10°C.[32]
In the CAT-promoted reduction of hydroperoxides process, CAT reacts very efficiently with H_2O_2 to form water and molecular oxygen and with H donors such as methanol, ethanol, formic acid, or phenols with peroxidase activity (Scheme 3).

$$2\,H_2O_2 \longrightarrow 2\,H_2O + O_2$$

$$ROOH + AH_2 \longrightarrow H_2O + ROH + A$$

Scheme 3. Catalyzed reduction of hydroperoxides.

Table 2 reports the amounts of protein obtained from different purification steps, the relative activities and the number of purifications, where the capacities of DEAE-C can be compared with other known method of purification. Similar comparisons will be given in Tables 3 and 4.

Table 2. Purification of catalase from *Azolla*. Protein amount, recovery and purification

Purif. step	Total protein (mg)	Total activity (u)	Specific activity (u/mg)	Recovery (%)	Purif. (fold)
Supernatant	430	121	0.28	100	1
Dialyzate	92	82	0.89	67.7	3.1
DEAE-C	87	52	1.67	42.9	5.9
Sephadex[a]	1.2	17.2	14.3	14	51.07

[a] G-75.

The enzyme was purified about 51.07-fold, with a final specific activity of 14.3 U/mg. The overall recovery of the purification was 14%. The molecular mass of purified catalase was 55 kDa as shown in Figure 7.

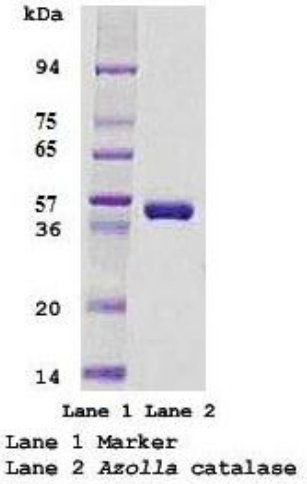


Figure 7. Molecular mass of purified catalase. [32]

Another example regards proteases that catalyze the cleavage of peptide bond in proteins affording smaller fragments such as small peptides and/or amino acids. They are distributed broadly in nature and a wide variety of microorganisms. Proteases are usually divided into two groups, exopeptidases or endopeptidases, depending on their site of hydrolysis. A proteases classification was attempted on the basis of the structural features of enzyme active center, rather than origin, specificity, or physiological action. It was preferred to divide them into four classes based on the type of functional group present at the active site and their mechanism of action: serine protease, aspartic protease, cysteine/thiol protease, and metalloprotease.

Proteases are extensively used in a variety of industries, including detergent, leather, pharmaceuticals, food, textile, bakery, soy-processing, peptide synthesis, and X-ray film. An ammonium sulfate precipitation of fermentation broth produced by *Bacillus subtilis* FBL-1 resulted in 2.9-fold increase of specific protease activity (Table 3). An eluted protein fraction from the column chromatography using DEAE-C and Sephadex G-75 had 94.2- and 94.9-fold higher specific protease activity, respectively.[33-35]

The enzyme was purified 94.89-fold with a yield of 2.3% from the crude extract, and the specific activity was increased to 3378.1 U/mg-protein.

Table 3. Purification step for the proteolytic enzyme from *Bacillus subtilis* FBL-1

Purif. step	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Recovery (%)	Purif. (fold)
Crude Extr.	7073.0	251794.5	35.6	100	1
$(NH_4)_2SO_4$ (30-80%)	175	17795.3	101.5	7.1	2.85
DEAE-C	3.9	12937.1	3352.4	5.1	94.17
Sephadex[a]	1.7	5669.9	3378.1	2.3	94.89

[a] G-75.

Glucose Isomerase (GI) is a microbial enzyme of immense commercial significance. The enzyme is used in large quantity for the production of high fructose corn syrup (HFCS), which is widely used in the United States and Japan as an alternative to sucrose or inverted sugar in the pharmaceutical, food, and beverage industry. GI produced from *Streptomyces albaduncus* was purified to homogeneity by ammonium sulphate precipitation, followed by ion exchange DEAE-C chromatography, and finally on DEAE-Sephadex A-50 chromatography. The MW of the purified enzyme was estimated to be 54 kDa by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE, Figure 8).

The purification steps of GI enzyme are summarized in Table 4.[36] The final preparation had 10.5% activity recovery and approximately 13.3-fold purification.

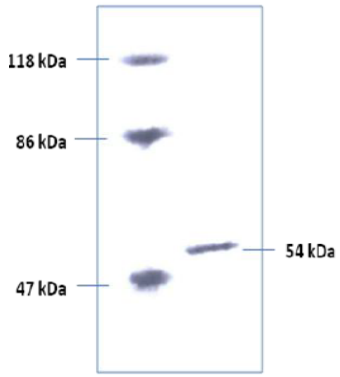


Figure 8. SDS-PAGE of purified GI (20 µg) produced by *S. albaduncus*. Lane 1: Molecular mass standards (β-galactosidase, 118 kDa; Bovine serum albumin, 86 kDa; ovalbumine, 47 kDa). Lane 2: purified GI enzyme produced by *S. albaduncus*.[36]

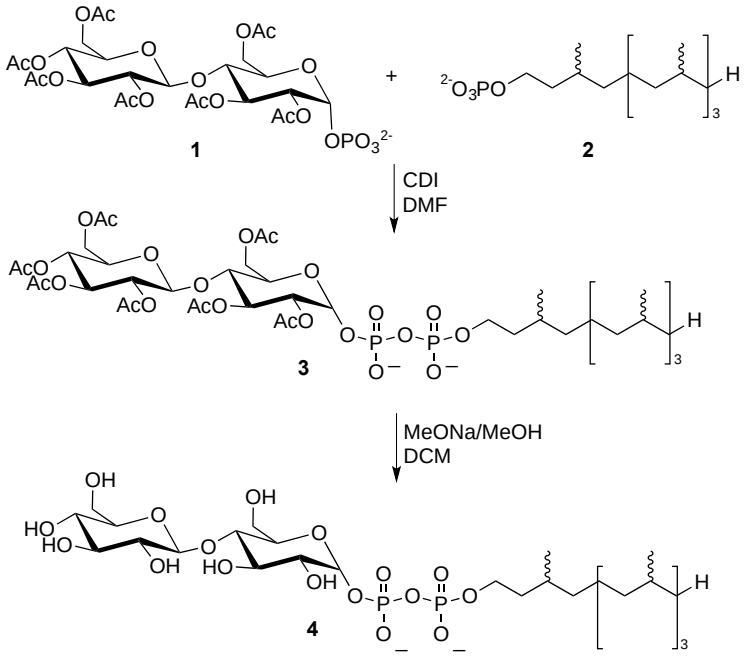
Table 4. Purification steps of GI enzyme from *S. albaduncus* culture

Purif. step	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Recovery (%)	Purif. (fold)
Crude Enz.	2728	4736	1.7	100	1
$(NH_4)_2SO_4$ (70-90%)	342	2682	7.8	56.6	4.6
DEAE-C	122	1486	12.2	31.4	7.2
Sephadex[a]	22	498	22.6	10.5	13.3

[a] A-50.

In 2011 Barrios and co-workers[37] showed that the polyisoprenyl-pyrophosphate-linked cellobiose, a natural acceptor of the α-1,3-annosyltransferase (AceA) from *Acetobacter xylinum*, is able to transfer mannose from GDP-mannose during the assembly of the heptasaccharide repeat unit of the exopolysaccharide acetan. Phytanyl α-D-cellobiosyldiphosphate **4** was previously synthesized by condensation of hepta-O-acetyl-α-D-cellobiosylphosphate **1** with phytanylphosphate **2**. Scheme 4 reports a two steps modified synthesis of **4**; compound **4** was obtained by *O*-deacylation of **3** with MeONa/MeOH in DCM solution and a purification step by anionic interchange chromatography was introduced. Thus, the crude *O*-deacetylated compound was fractionated on a DEAE-C column in the acetate form by an ammonium formate gradient. Compound **4** was isolated as the triethylammonium salt in an excellent yield (80%).

As seen from the above reported examples, DEAE-C chromatography represents a valuable and reliable methodology. We wish to summarize in Table 5 the cases of some isolated enzymes reported in recent literature, purified on this type of stationary phase. The extraction and purification of catechol 2,3-dioxygenase from *microalgae*[38] could be obtained from the crude enzyme solution by eluting on a DEAE 52 column using a buffer solution of NaCl. The cyclomaltodextrin glucanotransferase belongs to α-amylase family; it is a unique enzyme that degrades starch into cyclic oligosaccharides called cyclodextrins, which have numerous applications in various industries such as pharmaceutical, textile, agricultural, cosmetics, etc.[39] β-CGTase was isolated from *Bacillus flexus SV 1* and purified by starch adsorption followed by DEAE-C column chromatography which resulted in a fold purification of 6.1, with a yield of 44.07%.



Scheme 4. Synthesis of α-D-cellobiosyldiphosphate **4**.

Cerrena unicolor is a novel fungal source of highly active extracellular laccase that is currently used in industry. Chronic lymphocytic leukemia (CLL) is the most commonly observed adult hematological malignancy in Western countries. Despite the fact that recent improvements in CLL treatment have led to an increased percentage of complete remissions, CLL remains an incurable disease. *C. unicolor* was separated using anion exchange chromatography on DEAE-C/Sepharose and Sephadex G-50 columns. *C. unicolor* was able to

significantly induce cell apoptosis, and may represent a novel therapeutic agent for the treatment of various hematological neoplasms.[40]

β-Galactosidase was extracted by eight solutions from apricot fruit (*Prunus armeniaca kaisa*), purified in several protein purification steps and characterized biochemically. It was found that 10% sodium chloride at pH = 5 was the best solution for extraction the enzyme and that 20-60% ammonium sulfate saturation was the best method for partially purification of enzyme with a purification fold 4.31 and enzymatic recovery 61.6%. The final step was the purification by ion-exchange chromatography column using DEAE-C.[41] It is worth noting that ion exchange chromatography of β-galactosidase produced from local apricot using DEAE-C column equilibrated with potassium phosphate buffer pH = 7; lactase was eluted by a linear gradient of sodium chloride from (0.2-1.0)M. At variance, optimum pH of apricot β-galactosidases was estimated by incubation purified enzyme prepared in buffer solutions with various pH ranging from 3-8.

Tyrosinase inhibition is one major strategy used to treat hyperpigmentation. The results obtained from biological assays showed that *Glycyrrhiza glabra, Vetiveria zizanioides, Rosa indica* possessed anti-tyrosinase properties, with potential application in medical cosmetology;[42] the extracts were purified on DEAE-C. Pullulanase is a polymer synthesized by the yeast like fungus *Aureobasidium pullulans*. It is a linear α-D-glucan, built of maltotriose subunits, that has been isolated and purified from white edible mushrooms by ammonium sulphate precipitation (20-70%) followed by ion exchange chromatography (DEAE-C) and gel filtration (Sephadex G 75-120), with final yield (20%) and purification fold (17.8).[43]

Arginase, one of the urea-cycle enzymes, is a binuclear manganese cluster metallo-enzyme that catalyzes the hydrolysis of L-arginine to L-ornithine and urea. There is no information about its isolation and purification from camel liver; the method led to the purification to homogeneity using heat denaturation followed by ammonium sulphate precipitation with a combination of DEAE-C.[44]

An α-amylase inhibitor was isolated from the rhizome of *Cheilocostus speciosus* and was purified using DEAE-C anion exchange chromatography followed by gel filtration using Sephacryl-S-200 column. The purity and molecular mass of the purified inhibitor was determined by SDS-PAGE and LC-MS, respectively. The inhibition activity of the α-amylase inhibitor was stable and high at optimum pH 6 (52.2%) and temperatures of 30-40 °C (72.2%).[45]

The purification and characterization of a stable protease activity isolated from *Fasciola hepatica* adult worms were obtained by employing acetone precipitation (40-60%) followed by a gel filtration through Sephadex G-100 and DEAE-C ion exchange column. Through a three-step purification protocol, the enzyme was purified 11-fold with a specific activity of 1893.9 U/mg and 31.5% recovery. After the final ultrafiltration step, the purification fold was increased up to 13.1 and the overall activity yield reached a rate of 18.8%.[46]

A novel transglutaminase (MsTGase) from *Mythimna separata* larvae was found to display biochemical property and enzymatic catalytic activities and hence investigated. For this reason is was separated and purified; MsTGase was obtained chromatographically by the precipitation of Sephadex G-100 gel and DEAE-C-52 ion-exchange column with 48-fold purification and a reproducible yield of approximately 12%.[47]

It is known the ability of *E. coli* isolates to produce alkaline phosphatase enzyme (ALP). ALP can be extracted; the crude extract activity and specific activity was 1.86 unit/ml and 20.44 unit/mg protein, respectively. The enzyme purified by precipitating with ammonium sulfate (50-75%) saturation then using ion-exchange chromatography in DEAE-C ionic exchange column and gel filtration chromatography by using sepharose-6B gel filtration column, to obtained pure ALP enzyme with specific activity 272.73 unit/mg, with 13.34 fold purification and yield of enzyme 56.45%. The *in vivo* investigation for the effect of ALP enzyme on the infection with *P. aeruginosa* was done using BLBC mice. The result shows that ALP degreased the number of bacteria in liver, spleen and lung with significant differences ($P<0.05$).[48]

Table 5. Isolated enzyme by DEAE-C chromatography

Isolated enzymes	Natural Sources	Activities	Ref.
Catechol dioxygenase	Microalgae	Degradation of aromatic compounds	38
β-Cyclomaltodextrin glucanotransferase	*Bacillus flexus* SV 1	Pharmaceutical industries	39
Laccase	*Cerrena unicolor*	Antitumor activity against leukemic cells	40
β-Galactosidase	Prunus armeniaca kaisa	Lactose intolerance treatment	41
Mushroom Tyrosinase	*Glycyrrhiza glabra* *Vetiveria zizanioides* *(Rosa indica)*	Anti-Tyrosinase	42
Chitinase	*Aspergillus griseoaurantiacus*	Antifungal	43
Arginase	Camel liver cytosol	L-Arginine hydrolysis	44
Proteinaceous fungal α-amylase	Rhizome of Cheilocostus speciosus	α-amylase inhibitor	45
Cysteine protease	Liver fluke *Fasciola hepatica*	Specific epitope for the serodiagnosis of fascioliasis disease	46
Transglutaminase	Mythimna separata larvae (Noctuidae, Lepidoptera)	TGase	47
Alkaline phosphatase	*Pseudomonas aeruginosa*	Antibacterial	48
Amyloglucosidase	Thermophilic *Endomycopsis fibuligera*	Starch hydrolysis	49
Xanthine oxidase	Liver of the water buffalo *Bubalus bubalis*	Detecting reagent in many diagnostic kits	50
Beta-lactamase	Staphylococcus sciuri	Antimicrobial	51
Novel metalloprotease	Fruit bodies of *Oudemansiella radicata*	Proteolysis	52

An investigation on purification and characterization of amyloglucosidase enzyme from *Endomycopsis fibuligera* by fermentation of sago starch showed that this enzyme can be produced by fermenting sago starch in a medium containing *E. fibuligera*. Crude enzyme was obtained by centrifuging the medium cultures containing *E. fibuligera*. Purification by DEAE-C and Sephadex G-75 column chromatography produced three and one fractions with purity 17.4 and 22.5 times, respectively, compared to the crude extract enzyme. The amyloglucosidase activities was strongly increased by addition of Co^{2+} and Mn^{2+} ions, whereas the activities were weakly decreased by addition of K^{+}, Mg^{2+}, and Fe^{3+} ions.[49]

Xanthine oxidase (XO) is an important enzyme with broad medical applications as detecting reagent in many diagnostic kits. In a study, buffalo liver xanthine oxidase (BLXO) was purified to homogeneity by acetone precipitation and chromatography on DEAE-C column with a specific activity of 7.2 units/mg protein which represents 31.3 folds.[50]

Beta-lactamase (EC 3.5.2.6) was isolated and purified from two clinical isolates of *Staphylococcus sciuri* and *Klebsiella pneumoniae* by several steps included precipitation with ammonium sulphate at 80% saturation, DEAE-C and gel filtration on sephadex G-200 column. The enzyme from *S. sciuri* was more stable than that of

K. pneumoniae. The optimal pH values were 7.0 and 6.0 from *S. sciuri* and *K. pneumoniae,* respectively. The best concentrations of penicillin G were 400 μg/ml and 500 μg/ml for the enzyme from *S. sciuri* and *K. pneumoniae*. The increase in the enzyme concentration resulted in continuous increase in its activity from both bacteria.[51]

A 39-kDa metalloprotease was purified from a rare edible mushroom with health-promoting activities, *Oudemansiella radicata*, using a purification protocol, which entailed anion exchange chromatography on DEAE-C. Some peptide sequences were obtained by LC-MS/MS analysis. The protease was purified 79-fold and demonstrated a specific protease activity of 2.42 U/mg. The activity of the protease was inhibited by Cd^{2+}, Hg^{2+}, Cu^{2+}, Pb^{2+} and Fe^{3+} ions, but was enhanced by K^{+}, Mn^{2+} and Fe^{2+} ions. The marked suppression of the protease activity by EDTA indicates that the protease is a metalloprotease.[52]

5. Environmental Applications

In this section we will not give an exhaustive picture of the environmental applications of DEAE-C; we will concentrate the attention on one although widespread aspect regarding the ambient protection. The wastewater from industries is rich in compounds that are toxic and detrimental to different life forms including humans. To give an example with specific and noteworthy consequences on the ambient quality, dye industries often produce wastewater, harmful for the environment. Colour is usually the first contaminant to be recognized which affects the aesthetic merit, transparency and gas solubility of water bodies. The unused synthetic dyes in textile effluent mostly go untreated in the river and water bodies. Disperse dyes constitute the largest group of colorants used in the industry and are difficult to remove by chemical treatment. The processes involving physical and chemical treatment for decolorization of textile wastewater have numerous operational problems, and involve high cost. On the other hand, most of the synthetic dyes are xenobiotic compounds, which are poorly removed by the use of conventional biological aerobic treatments. The preparation and application of DEAE-immobilized in decolorization of synthetic dyes was thoroughly investigated. The data here reported reveals the effectiveness of the immobilized peroxidase in sustainable dye colour removal.

Pointed Gourd (*Trichosanthes dioica*) is a member of the cucurbit family and an important vegetable crop in India, which is also widely grown in West Bengal. Fungal diseases are a major constraint to cultivation of Pointed Gourd (Figure 9).

Figure 9. Pointed Gourd (*Trichosanthes dioica*).

Pointed Gourd contains Peroxidase (PGP) that expresses a remarkable activity and can be repeatedly used and stably stored for long periods. The system is developed with a cheaper biocatalyst that is quite

effective in treating dyes continuously in a small laboratory reactor. The enzymes in soluble form cannot be exploited on large scale due to their limitations of stability and reusability. Consequently, the use of immobilized enzymes has significant advantages over soluble enzymes. In the near future, cost effective, eco-friendly technologies based on the enzymatic approach for treatment of dyes present in the industrial effluents/waste water will play a vital role.

Table 6. Re-usability of DEAE-immobilized PGP in decolourization of dye and dye mixture

No of uses DEAE-PGP complex	Peroxidase activity (%)	DR19 (λ 495nm)[a]	DR19 and DB9 (λ 460nm)[a]
1	89.7	91.5	82.2
2	78.8	87.4	80.6
3	69.7	74.6	72.7
4	58.4	69.8	65.4
5	50.7	64.9	61.5
6	42.4	60.6	59.4
7	36.3	58.9	51.8
8	29.7	51.4	43.2
9	18.9	33.6	28.5
10	15.5	21.4	19.6

[a] Percent dye decolorization.

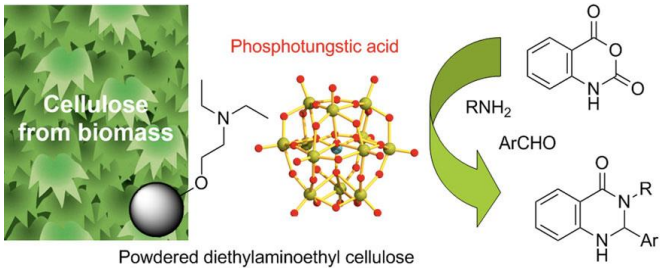
By immobilization using adsorption on DEAE-C, the apparent rate of enzyme inactivation was reduced, which allowed a significant reduction in enzyme requirements for treatment. This increases enzyme lifetime and represents a very significant saving in terms of treatment costs.[53]

Immobilized PGP was independently incubated with DR19 and mixture of dyes (DR19+DB9) at 40°C. Dye decolourization was determined after incubation period of 2 h. The immobilized enzyme was collected by centrifugation and stored in assay buffer at 4°C overnight. The similar experiment was repeated 10 times. Each value in Table 6 represents the mean for three independent experiments performed in duplicate and indicate re-usability of DEAE-immobilized PGP in decolourization of dye and dye mixtures; the expressed peroxidase activity declined progressively to 15.5% in the tenth use. Although PGP immobilized on DEAE-C was losing its activity over repeated uses, the authors believe that the decrease in efficiency of the enzyme after few cycles may be due to the binding of the active sites of the enzyme by the product produced during the enzymatic reactions.

The preparation and application of DEAE-immobilized Pointed Gourd peroxidase in decolorization of synthetic dyes was investigated. The data of the present work reveals the effectiveness of the immobilized peroxidase in sustainable dye color removal. The DEAE immobilized PGP expressed remarkable peroxidase activity, can be repeatedly used and stably stored for long periods. The system is developed with a cheaper biocatalyst that is quite effective in treating dyes continuously in a small laboratory reactor.

A Chinese research group[54] disclosed a method for recovering sericin in silk-reeling industrial waste water, including treating silk-reeling industrial wastewater by filtering to remove impurities, resin adsorption, elution, collecting, to obtain sericin solution. The resin used in resin adsorption process is DEAE-C 52 ion exchange resin. The invention has simple technique, less equipment investment, reusable choice of resin, easy

to achieve continuous production and, for these reasons, suitable for industrial promotion. The obtained sericin has higher purity, has no introducing harmful substances and can be used for food and other industries. A method for preparing fiber-based phosphatidic acid flame retardant has been proposed and patented; it comprises the steps of carrying out reaction between DEAE-C and 2-hydroxy-6-methoxy-benzaldehyde in solvent to get an intermediate material, then carrying out reaction between the intermediate and phosphorus oxychloride ($POCl_3$), and processing to obtain the final product. The phosphorus-based flame retardant has high contents of nitrogen and phosphorus, high molecular weight, excellent flame retardancy and being environment-friendly, and can be used for flame retardance of polyvinyl chloride, polystyrene, textiles and coatings. The preparation method is simple and suitable for industrial use.[55]



Scheme 5. Biopolymer-supported solid acidic catalyst for the synthesis of 2,3-dihydroquinazolin-4(1*H*)-ones from isatoic anhydride, amines, and aldehydes.[58]

In 2016 Farrukh and *et al.* reported that ciprofloxacin-encapsulated polymeric nanoparticles displayed a remarkable antibacterial potential against a variety of gram-positive and -negative pathogenic bacteria. Ciprofloxacin-encapsulated nanoparticles of DEAE-C were prepared by the multiple emulsion solvent evaporation method. Ciprofloxacin-encapsulated nanoparticles showed superior effectiveness to inhibit the growth of bacteria *in-vitro*.[56] Biodegradable and biocompatible polymers such as DEAE-C are used in combination with iron, which contributes with its magnetic characteristics to the nanocomposite. The nanoparticles produced could be used as carriers for releasing chloramphenicol at the targeted site for sustained release.[57]

We conclude with a couple of examples where DEAE-C is derivatized.

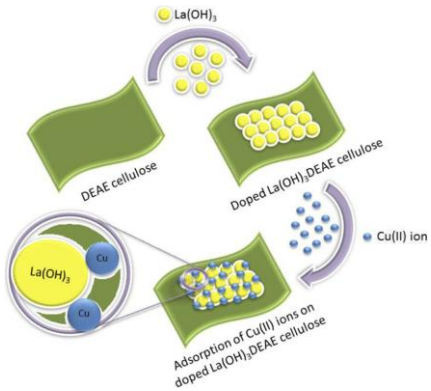


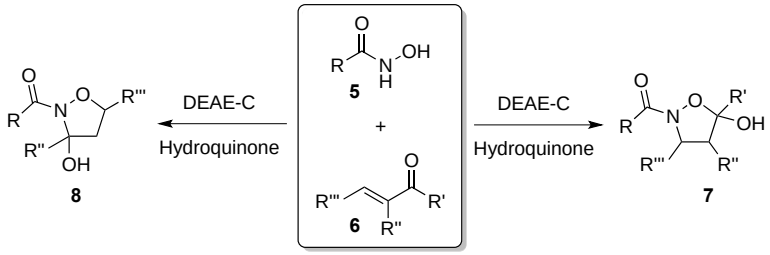
Figure 10. Schematic view of adsorption on modified DEAE-C.[59]

Finely powdered DEAE-C can be prepared through a bottom-up synthetic method using NaOH/urea aqueous solution as solvent. Phosphotungstic acid anchored on the powdered DEAE-C can be used as a

biopolymer-supported solid acidic catalyst for the synthesis of 2,3-dihydroquinazolin-4(1*H*)-ones from isatoic anhydride, amines, and aldehydes (Scheme 5). The catalyst could be easily recovered by simple filtration and reused several times with minor decreases in the reaction yields.[58] A simple, reliable, and rapid method based on modifying the cellulose surface by doping it with different percentages of lanthanum hydroxide [e.g., 1% $La(OH)_3$-cellulose (LC), 5% $La(OH)_3$-cellulose (LC2), and 10% $La(OH)_3$-cellulose (LC3)] was proposed by Marwan and co-workers. This modified cellulose became a selective marker for detection of copper Cu(II) in aqueous medium (Figure 10).[59]

6. DEAE-C in Organic Synthesis

We have seen as according to literature DEAE-C has been used as a sort of functionalized cellulose, commonly in ion exchange chromatography, as a kind of anion exchange fiber. DEAE-C is widely employed in the isolation and purification of polysaccharides, peptides, nucleotides, etc. However, its applications in catalysis and specifically in organic chemistry have not been thoroughly explored. Nevertheless, some suggestions can be found in literature, even in this field.



Scheme 6. Synthesis of 3- and 5-hydroxyisoxazolidines **7** and **8** by reaction of hydroxylamines **5** and α,β-unsaturated carbonyl compounds **6**.

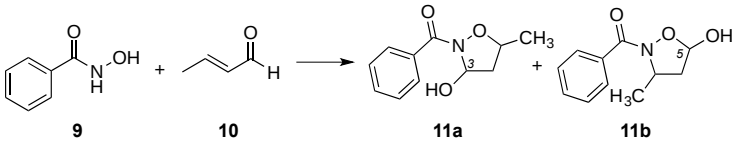
The reactions of N-substituted hydroxylamines with alkenals served as a method for the synthesis of 3- and 5-hydroxyisoxazolidines **7** and **8**. As these compounds have a hemiaminal or hemiacetal fragment in their structure, they are in priciple unstable and in most cases, only products of the elimination of a water molecule can be isolated.[60] The use of DEAE-C as heterogenous catalyst allowed to set-up an efficient method for synthesizing previously undescribed 3- and 5-hydroxyisoxazolidines **7** and **8** by reaction of hydroxylamines **5** and α,β-unsaturated carbonyl compounds **6** yielding to the desired products with aryl substituents (Scheme 6).

A typical and remarkable example deals with the reaction of the benzohydroxamic acid **9** and crotonaldehyde **10** in methanol solution; the reaction mixture composition is 3-hydroxyisoxazolidine **11a** (98%) and 5-hydroxyisoxazolidine **11b** (2%). The adsorption on cellulose causes an acceleration of the reaction with the complete conversion of the reagents into the desired 2-benzoyl-3-hydroxy-5-methyl isoxazolidine. Under conventional conditions (0 °C, methanol solution, DEAE-C catalysis) the product is achieved only after prolonged stirring (5-7 h) and the yield after isolation and purification is about 60%. The same conversion in an adsorbed state with SiO_2 at 20 °C is complete within 30-40 minutes and the yield is 90%.

Quite surprisingly it turned out that the isolated product structure (3- or 5-isomer) depends on the type of adsorbent (see Table 7). It may be supposed that these results can be explained by the difference in basic properties of adsorbents. It was also found that different diastereomers are formed in equal amounts in the

case of 2-benzoyl-3-hydroxy-5-methylisoxazolidine **11a**, but only one of the diastereomeric pair, the 2-benzoyl-5-hydroxy-3-methylisoxazolidine **11b**, is formed in the presence of DEAE-C.

Table 7. Effect of adsorbent on the composition of the reaction products



Adsorbent	Total yield %	3-OH isomer %	5-OH isomer %
Neutral alumina	70-75	80-85	20-15
Acid alumina/KF	70	75-80	25-20
Acid alumina	60-70	85-80	15-20
Florisil	50-70	85-90	15-10
Inerton AW	73	68	32
Silica gel	80-90	100	0
Cellulose	43	75	25
CM-Cellulose	41	70	30
DEAE-C	95-98	0	100
TEAE-C	98	0	100

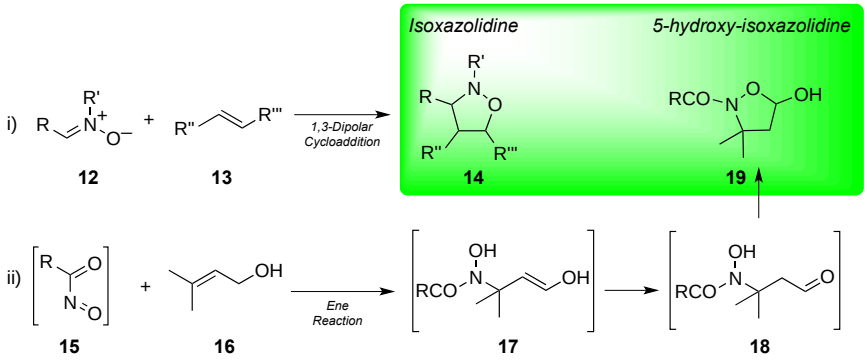
Further investigations of the reaction on DEAE-C as adsorbent showed that for all starting hydroxamic acids and carbonyl compounds (acrolein, crotonaldehyde, etc.), only adduct **11b** form in good yield (overnight incubation). The inversion of addition direction in comparison with that in methanol solution was observed for X=H, Br and Ar=$C_6H_2(CH_3)_3$. On the other hand, when the reaction time was reduced to 1-2 h, a mixture of the 3- and 5- isomers was obtained (see Table 8). Increasing the reaction time led to the transformation of 3-hydroxyisoxazolidines into the 5-isomer on the surface of an adsorbent. Nevertheless, product **11** are not formed even under prolonged stirring in solution (20-40 days) for X=NO_2, Br and Ar=$C_6H_2(CH_3)_3$. These data support the fact that the formation of 3-hydroxy-isoxazolidines is reversible and DEAE-C catalyzes not only the addition reaction, but also the transformation of the kinetic product 3-hydroxy-isoxazolidine into the thermodynamically more stable 5-isomer.[61]

Table 8. Reaction conditions, reaction time and **11a,b** ratio (%)

Reaction condition	Time	**11a**	**11b**
MeOH solution	5 h	98	2
MeOH solution	30 days	95-98	2-5
Adsorbed state, silica gel	30 min	100	0
Adsorbed state, DEAE-C	1 h	60	40
Adsorbed state, DEAE-C	6 h	4	96

These uses of DEAE-C in the synthesis of isoxazolidines somewhat recall for the known methods to prepare these saturated heterocycles. Two are the main approaches that can be found in recent literature.

The first is the classical 1,3-dipolar cycloaddition of nitrones **12** to C=C double bonds of alkenes **13** (Scheme 7). This is quite a common and reliable way to obtain isoxazolidines **14**, variably substituted on the nitrogen atom as well as in the positions 3, 4 and 5 of the heterocyclic ring.[62]

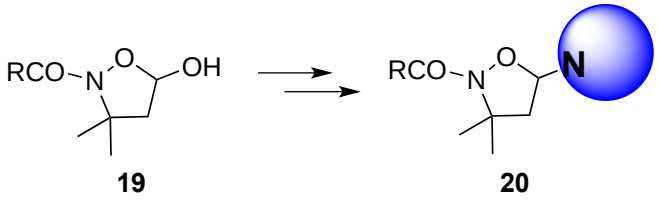


Scheme 7. General approaches to Isoxazolines and 5-hydroxy-isoxazolidines.

Reliable methods are also known to perform the cycloaddition in a diastereoselective way.

The second methodology is offered by the use of the chemistry of nitrosocarbonyl intermediates **15**, fleeting compounds that are usually generated upon oxidation of hydroxamic acids or mild oxidation of nitrile oxides. Besides their ability to add to dienes in a hetero Diels-Alder cycloaddition reactions, they also undergo ene reaction under very mild conditions to give ene adducts. If the ene partner is an allylic alcohol of type **16**, the ene reaction can occur on the more substituted carbon atom of the C=C unsaturation affording the enol **17** as primary adduct, that immediately is transformed into the aldehyde **18**. Once formed, this latter spontaneously cyclize to the 5-hydroxy-isoxazolidine **19**.[63]

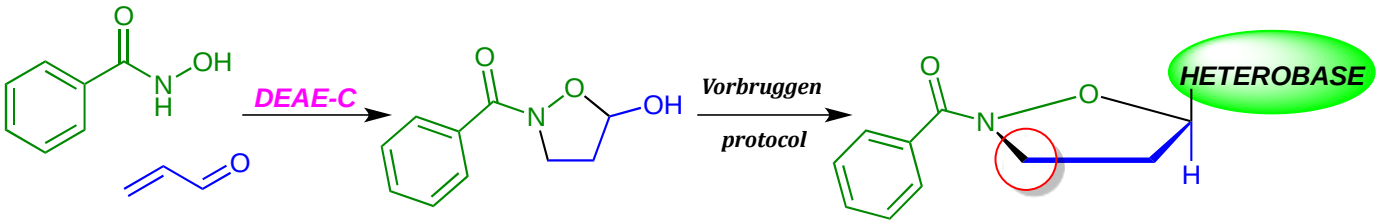
5-Hydroxy-isoxazolidines are quite interesting from the synthetic point of view as the hydroxyl group in 5 can be replace by nucleophilic compounds.[64] In our group we actively pursued this methodology by adapting the Vorbruggen protocol to functionalize these isoxazolidine with heterobases to prepare *N,O*-nucleoside analogue with potential antiviral and apoptotic activities (Scheme 8).[65]



Scheme 8. General Vorbrüggen protocol to functyionalize hydroxyl-isoxazolidines with heterobases to prepare *N,O*-nucleoside analogues.

The synthetic protocol represented in Scheme 8 is a valuable alternative to the nitrone/olefin cycloaddition reaction but finds the greatest limitation into the requirements on the alkene side to be used in the presence of nitrosocarbonyl intermediates. These highly reactive species like highly substituted C=C double bonds and it is quite uncommon to find allylic alcohols with these features. Being one of the starting materials for nitrosocarbonyl synthesis the hydroxamic acids, the same material used under DEAE-C catalysis, the use of this resin under the conditions above presented represents the opportunity to extend the isoxazolidine synthesis to a lager variety of reactants, both on the hydroxamic acid and the alkene sides.

Recently, we successfully applied the DEAE-C-catalyzed protocol, properly tuned up to be adapted to our purposes, to the synthesis of 5-hydroxy-isoxazolidinic heterocycles, easily prepared from hydroxamic acids and suitable α,β-unsaturated aldehydes or ketones in good yields. The synthesized products were efficiently derivatized with representative heterobases for the preparation of nucleoside analogues with a methylene group in the position 3 of heterocyclic ring and e benzoyl group at the nitrogen atom, suitable for biological evaluation and SAR analysis (see Scheme 9).[66]



Scheme 9. Vorbruggen protocol to nucleoside analogues

Our interest in these and other methodologies is actively pursued and hopefully further investigation could offer new pathway to isoxazolidines and other applications of DEAE-C catalysis in organic chemistry.

7. Perspectives

Other applications of DEAE-C catalysis in organic chemistry are at the moment unknown at the best of our knowledge. Surely, being DEAE-C a solid compound, its applications as a solid catalyst in solvent-free organic reactions represent an intriguing opportunity to perform organic reaction in eco-friendly conditions. Moreover, the solid form of the catalyst would offer an easy work-up procedure allowing for the isolation and fast purification of the final products of a reaction.

As a common ammonium salt, it can be used as a catalyst in organic reaections, such as esterification, hydrolysis, electrophilic addition and substitutions, and many others where a mild acid catalyst is required.

Linked to this latter point, being a mild catalyst, side reactions can be avoided or reduced in their impact on the chemical yields and in general in the outcome of a reaction, hopefully directing the reaction towards the desired product, only.

As a cellulose derivative, DEAE-C is an eco-friendly and easily disposable catalyst or reaction promoter and offers an easy use to the synthetic chemist.

Exploration of all these fields could be of great advantage in improving the performance of DEAE-C besides the simple use in chromatographic technologies.

Conclusions

Upon reviewing the uses of DEAE-C, it arises that this positively charged resin having a cellulose core finds the principal applications in purification processes. The enormous literature dealing with DEAE-C focuses the attention on chromatographic separations, purifications and fractioning of macromolecules. As hereby exposed, proteins, enzymes as well as polysaccharides of different structures purification protocol are

accounted in recent literature. Moreover, the capability of DEAE-C to immobilize polar (charged) organic molecules such as dyes and in general coloured materials makes the use of this resin quite interesting in environmental applications, specifically for the treatment of waste waters.

Very few examples can be found regarding the organic synthesis applications. It is difficult to say if this is due to the failure of attempts made to use DEAE-C or just to forgetfulness or distraction of the organic chemistry investigators. The reported examples deal with the synthesis of isoxazolidines and the selectivity results could be quite interesting a worth of further investigation, as the methodology seems to be a valuable alternative especially for the opportunity that offers to extend the application to a variety of hydroxamic acids and unsaturated carbonyl compounds. However, the authors that proposed this method did not further investigated the protocol.

The aim of this review is to reset the attention of the reader to the extension of the use of DEAE-C to organic reactions, possibly not only devoted to the preparation of heterocycles but hopefully also to other fields. In fact, being DEAE-C an ammonium salt, it can be considered as a potential mild acid catalyst or a proton donor and these features could in theory catalyze standard acid-catalyzed organic reactions. In addition, the resin nature of DEAE-C could suggest the way to perform organic reactions in the solid state; an interesting methodology, for example, is that of the fixed-bed reactor filled with the catalyst, through which the reagent flow, leaving at the end the desired product. The same criterion is applicable to flow-chemistry reactors.

All these suggestions and others that can be considered could give the resin DEAE-C a new life and perspective in organic synthesis and greener method for the preparation of organic compounds; being a cellulose-based material, there is motivation to favor the use of DEAE-C in place of various petroleum-based products, with a long-term goal of decreasing environmental impacts.

Acknowledgements

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References

1. Peuravuori, J.; Pihlaja, K. *Anal. Chim. Acta* **1998**, *363*, 235-247. https://doi.org/10.1016/S0003-2670(98)00136-6
2. Reichert, W. M.; Anderson, E. D. *ECS Transactions* **2016**, *75*, 685-691. https://doi.org/10.1149/07515.0685ecst
3. Nelson, D. L.; Cox, M. M. *Lehninger's Principle of Biochemistry,* 2005, 4th Ed. W. H. Freeman and Co.
4. Janson, J. C. *Protein Purification Principles, High Resolution Methods, and applications, 3rd Ed., Wiley series in Methods of Biochemical Analysis*, 2011, p. 54. https://doi.org/10.1002/9780470939932
5. Mayhew, S. G.; Howell, L. G. *Anal. Biochem*. **1971**, *41*, 466-470 and reference 2 therein. https://doi.org/10.1016/0003-2697(71)90166-7

6. Soukhtehzari, S.; Rasaee, M. J.; Javanmardi, M. *Inter. J. Pept. Res. Ther.* 2018. https://doi.org/10.1007/s10989-018-9719-4
7. Gayatridevi, S.; Smd, A.; Jayalakshmi, S. K.; Sreeamulu, K. *The Bioscan* **2016**, *11*, 73-77.
8. Rodrigues, J. A. C.; de Queiroz, I. N. L.; Quinderé, A. L. G.; Benevides, N. M. B.; Tovar, A. M. F.; de S. Mourão, P. A. *Acta Scientiarum Biol. Sci.* **2016**, *38*, 7-15. https://doi.org/10.4025/actascibiolsci.v38i1.28257
9. Queiroz, I. N. L.; Wang, X.; Glushka, I. N.; Santos, G. R. C.; Valente, A. P.; Prestegard, J. H.; Pomin, V. H. *Glycobiology* **2015**, *25*, 535-547. https://doi.org/10.1093/glycob/cwu184
10. Xu, P.; Yuan, R.; Hou, G.; Li, J.; Ye, M. *Appl. Biochem. Biotechnol*. **2018**, *185*, 541-554. https://doi.org/10.1007/s12010-017-2668-0
11. Gao, J.; Jiang, F.; Zhai, G. *Macromol. React. Eng.* **2016**, *10*, 269-279. https://doi.org/10.1002/mren.201500067
12. Xu, C.; Qin, N.; Yan, C.; Wang, S. *Food Funct*. **2018**, *9*, 2644-2652. https://doi.org/10.1039/C7FO01921A
13. Tung, C. H.; Lin, C. C.; Tung, C. C.; Chen, S. F.; She, F.; Lu, T. J. *J. Food Drug Anal*. **2018**, *2*, 1045-1053. https://doi.org/10.1016/j.jfda.2017.12.004
14. Chen, G. T.; Fu, Y. X.; Yang, W. J.; Hu, Q. H.; Zhao, L. Y. *Int. J. Biol. Macromol*. **2018**, *107*, 2150-2156. https://doi.org/10.1016/j.ijbiomac.2017.10.090
15. Hu, X.; Li, W.; Sun, Y.; Fan, M. *Adv. J. Food Sci. Tech.* **2016**, *12*, 326-330. https://doi.org/10.19026/ajfst.12.2968
16. Liu, Q.; Fang, J.; Wang, P.; Dub, Z.; Li, Y.; Wanga, S.; Ding, K. *Int. J. Biol. Macromol.* **2018**, *107*, 112-120. https://doi.org/10.1016/j.ijbiomac.2017.08.154
17. Liu, Q.; Ge, X.; Chen, L.; Cheng, D.; Yun, Z.; Xu, W.; Shao, R. *Int. J. Biol. Macromol.* **2018**, *107*, 2262-2268. https://doi.org/10.1016/j.ijbiomac.2017.10.095
18. Jahanbin, K. *Int. J. Biol. Macromol.* **2018**, *107*, 1227-1234. https://doi.org/10.1016/j.ijbiomac.2017.09.100
19. Shehataa, A. N.; Abd El Aty, A. A.; Darwish, D. A.; Abdel Wahab, W. A.; Mostafa, F. A. *Int. J. Biol. Macromol*. **2018**, *107*, 990-999. https://doi.org/10.1016/j.ijbiomac.2017.09.071
20. Pan, X. X.; Tao, J. H.; Jiang, S.; Zhu, Y.; Qian, D. W.; Duan, J. A. *Int. J. Biol. Macromol*. **2018**, *107,* 9-16. https://doi.org/10.1016/j.ijbiomac.2017.08.130
21. Liu, Y.; Zhang, J.; Meng, Z. *Int. J. Biol. Macromol*. 2018, *109*, 1054-1060. https://doi.org/10.1016/j.ijbiomac.2017.11.093
22. Liu, Y.; Zhou, Y.; Liu, M.; Wang, Q.; Li, Y. *Int. J. Biol. Macromol.* **2018**, *112*, 326-332. https://doi.org/10.1016/j.ijbiomac.2018.01.132
23. Li, S.; Yang, G.; Yan, Y.; Wua, D.; Hou, Y.; Diao, Q.; Zhou, Y. *Int. J. Biol. Macromol.* **2018**, *111*, 660-666. https://doi.org/10.1016/j.ijbiomac.2018.01.015
24. Qu, H.; Yang, W.; Li, J. *Int. J. Biol. Macromol.* **2018**, *113*, 849-858. https://doi.org/10.1016/j.ijbiomac.2018.03.005
25. Han, M.; Sun, P.; Li, Y.; Wu, G.; Nie, J. *Int. J. Biol. Macromol*. **2018**, *108*, 1120-1127. https://doi.org/10.1016/j.ijbiomac.2017.12.109
26. Isnansetyo, A.; Lutfia, F. N. L.; Nursid, M.; Susidarti, R. A. *Pharmacogn. J.* **2017**, *9*, 14-20. https://doi.org/10.5530/pj.2017.1.3

27. Xie, J.; Zou, L.; Luo, X.; Qiu, L.; Wei, Q.; Luo, D.; Wu, Y.; Jiao, Y. *Int. J. Biol. Macromol*. **2018**, *114*, 520-528. https://doi.org/10.1016/j.ijbiomac.2018.03.124
28. Ma, Q.; Yuan, L.; Zhuang, Y. *Int. J. Biol. Macromol.* **2018**, *114*, 97-105. https://doi.org/10.1016/j.ijbiomac.2018.03.099
29. Palanisamy, S.; Vinosha, M.; Manikandakrishnan, M.; Anjali, R.; Rajasekar, P.; Marudhupandi, T.; Manikandan, R.; Vaseeharan, B.; Marimuthu Prabhu, N. *Int. J. Biol. Macromol.* **2018**, *116*, 151-161. https://doi.org/10.1016/j.ijbiomac.2018.04.163
30. Jahanbin, K.; Abbasian, A.; Ahang, M. *Carbohyd. Polym*. **2017**, *178*, 386-393. https://doi.org/10.1016/j.carbpol.2017.09.058
31. Zhang, T.; Xiang, J.; Zheng, G.; Yan, R.; Min, X. *J. Func. Foods* **2018**, *41*, 19-24. https://doi.org/10.1016/j.carbpol.2017.09.058
32. Susmitha, S.; Shyamala Gowri, R.; Meenambigai, P.; Ramitha, R.; Vijayaraghavan, R. *Int. J. Curr. Microbiol. Appl. Sci.* **2016**, *5*, 836-844. https://doi.org/10.20546/ijcmas.2016.502.095
33. Si, J. B.; Jang, E. J.; Charalampopoulos, D.; Wee; Y. J. *Biotech. Bioprocess Eng.* **2018**, *23*, 176-182. https://doi.org/10.1007/s12257-017-0495-3
34. Omprakash, K.; Du, B. *Int. Res. J. Sci. Eng*. **2015**, *3*, 47-50.
35. Daniel, D.; Subramaniyan, S.; Sandhia; G. S. *Int. J. Adv. Res.* **2016**, *4*, 1035-1046.
36. Yassien, M. A. M.; Jiman-Fatani, A. A. M.; Asfour, H. Z. *Afr. J. Microbiol. Res.* **2013**, *7*, 2682-2688.
37. Barrios, P.; Ielpi, L.; Marino, C. *Arkivoc* **2011**, *vii*:38-48. https://doi.org/10.3998/ark.5550190.0012.707
38. Chen, Q.; Li, J.; Liu, M.; Tao, L.; Guo, Q.; Mu, J. *Faming Zhuanli Shenqing* (2018) CN 107858334, A 20180330.
39. Reddy, S. V.; More, S. S.; Annappa, G. S. *Basic Microbiol*. **2017**, *52*, 974-981. https://doi.org/10.1002/jobm.201700270
40. Matuszewska, A.; Karp, M.; Jaszek, M.; Janusz, G.; Osińska-Jaroszuk, M.; Sulej, J.; Stefaniuk, D.; Tomczak, A.; Giannopoulos, K. *Oncology Lett.* **2016**, *11*, 2009-2018. https://doi.org/10.3892/ol.2016.4220
41. Al-Arrji, S. B.; Al-Hamadi, N. A. Int. J. Chem. Tech. Res. **2017**, *10,* 919-929.
42. Vanitha, M.; Soundhari, C. Int. J. Chemtech Res. **2017**, *10*, 1156-1167.
43. Shehata, A. N.; Darwish, D. A.; Masoud, H. M. M. *J. Appl. Pharma. Sci*. **2016**, *6*, 147-152. https://doi.org/10.7324/JAPS.2016.600123
44. Maharem, T. M. M.; Zahran, W. E.; Hassan, R. E.; Abdel Fattah, M. M. *Int. J. Biol. Macromol.* **2018**, *108*, 88-97. https://doi.org/10.1016/j.ijbiomac.2017.11.141
45. Balasubramanian, A.; Bhattacharjee, M.; Sakthivel, M.; Thirumavalavan, M.; Madhavan, T.; Nagarajan, S. K.; Palaniyandi, V.; Raman, P. *Int. J. Biol. Macromol.* **2018**, *111*, 39-51. https://doi.org/10.1016/j.ijbiomac.2017.12.158
46. Hemici, A.; Benerbaih, R. S.; Bendjeddou, D. *J. Chrom. B*, **2017**, *1068-1069*, 268-276. https://doi.org/10.1016/j.jchromb.2017.10.049
47. Zhang, L.; Rao, W.; Muhayimana, S.; Zhang, X.; Xu, J.; Xiao, C.; Huang, Q. *J. Biotech.* **2018**, *265*, 1-7. https://doi.org/10.1016/j.jbiotec.2017.10.018
48. Hashem, K. A.; Authman, S. H.; Mahdi, L. H. *The Pharma Innov. J*. **2016**, *5*, 32-36.
49. Ahmad, A.; Karim, H. *Indones. J. Chem.* **2016**, *16*, 302-307.

https://doi.org/10.22146/ijc.21147
50. Ibrahim, M. A.; Masoud, H. M. M.; Darwish, D. A.; Esa, S. S.; Zaahkouk, S. A. M. *J. Appl. Pharmac. Sci*. **2015**, *5*, 63-68. https://doi.org/10.7324/JAPS.2015.501110
51. El-Shora, H. M.; Al-Hayanni, H. S.; El-Shobacky, A. M. *Int. J. Curr. Microbiol. App. Sci.* **2017**, *6*, 927-941. https://doi.org/10.20546/ijcmas.2017.606.109
52. Geng, X.; Te, R.; Tian, G.; Zhao, Y.; Zhao, L.; Wang, H.; Bun, N. T. *Acta Biochim. Polonica* **2017**, *64*, 477-483. https://doi.org/10.18388/abp.2016_1431
53. Jamal, F.; Goel, T. *J. Bioproc. Biotech.* **2014**, 4-7.
54. Method for recovering sericin in silk-reeling industrial wastewater: Xiaoling, L.; Ruiting, J.; Mouming, Z.; Hongrui, J.; Yi, J.; Yuanxin, J. *Faming Zhuanli Shenqing* (2017) CN 106745878, A 20170531.
55. Method for preparing fiber-based phosphatidic acid flame retardant: Juguang, C. *Faming Zhuanli Shenqing* (2017) CN 106674356, A 20170517.
56. Ciprofloxacin loaded DEAE-C nanoparticles: Farrukh, M. A.; Gul, M. L.; K.-ur-Rahman, M. U.S. Pat. Appl.: (2016) US 20160106685, A1 20160421.
57. Drug entrapment efficiency and sustained drug release of chloramphenicol loaded polymeric-iron nanoparticles: Muhammad Akhyar, F.; Adarsh, S.; Khaleeq-ur, R. M. U.S. Pat. Appl.: (2016) US 20160101182, A1 20160414.
58. Li, S.; Zhang, Q.; Peng, Y. *Monatsh. Chem.* **2015**, *146,* 1859-1864. https://doi.org/10.1007/s00706-015-1475-y
59. Marwani, H. M.; Lodhi, M. U.; Khan, S. B.; Asiri, A. M. *Nanoscale Res. Lett*. **2014**, *9*, 466. https://doi.org/10.1186/1556-276X-9-466
60. Zelenin, K. N.; Motorina, I. A.; Svirdova, L. A.; Bazhan, I. P.; Ershov, A. Y.; Golubeva, G. A.; Bundel, Y. G. *Khim. Geterots. Soed.* **1987**, *9*, 1270-1275.
61. Motorina, I. A.; Svirdova, L. A.; Golubeva, G. A.; Bundel, Y. G. *Tetrahedron Lett*. **1989**, *30*, 117-120. https://doi.org/10.1016/S0040-4039(01)80339-7
62. Martin, J. N.; Jones, R. C. F. "Nitrones" in *Synthetic Applications of 1,3-Dipolar Cycloaddition Chemistry Toward Heterocycles and Natural Products*, Padwa, A.; Pearson, Ed., W. H.; Wiley: Hoboken, NJ, 2003; Cpt. 1, p. 1. https://doi.org/10.1002/0471221902.ch1
63. Memeo, M. G.; Quadrelli, P. *Chem. Rev*. **2017**, *117*, 2108-2200. https://doi.org/10.1021/acs.chemrev.6b00684
64. P. Quadrelli, M. Mella, S. Carosso, B. Bovio, *J. Org. Chem*. **2013**, *78*, 516-526. https://doi.org/10.1021/jo302346a
65. Quadrelli, P. *Modern Applications of Cycloaddition Chemistry*, Quadrelli, P. Ed.; Elsevier: Amsterdam, 2019; Cpt. 2, p. 85. https://doi.org/10.1016/B978-0-12-815273-7.00002-2
66. Aljaf, K. K.; Amin, A. A.; Hussain, F. H. S.; Quadrelli, P. *Arkivoc* **2020**, *vi*, 73-83. https://doi.org/10.24820/ark.5550190.p011.159

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