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Organic Polymer Monolith: Synthesis and Applications For Bioanalytical

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***Abstract*— High-speed separation, enrichment, digestion, as well as high-throughput analysis of biomolecules are very important in analytical and bioanalytical chemistry, biosciences, etc. for thesepurposes, currently a single piece of porous material so-called "monolith", Has been rapidly developed for Several decadesand nowadays holds an impressively strong position in separation science and further extended in other areas of chemistry ie media extraction and catalyst supports, Recently, we are Involved in development of analytical procedures for separation and accurate detection of biomolecules through the development of organic polymer-based Monoliths coupled with chromatographic systems. The addition of monolith columns as the stationary phase HPLC on the system has been proven to improve the performance of the system, since monolith possesses a number of advantages over conventional packed-column. Uniformity of bed with no end frits, higher permeability, and fast separation / reaction time are the main advantages of monolithic stationary phase. Reviews These Monoliths, the which were coupled into HPLC as well as CFFN HPLC-ICP / MS system, were applied to separation and Quantificationof ssDNA, dsDNA, methylated DNA, single nucleotide polymorphism (SNP), protein, peptide mapping, and enrichment of Phosphopeptide. So far, we have Monoliths prepared a reverse phase of poly (lauryl methacrylate-co-ethylene dimethacrylate)(Poly (LMA-co-EDMA)) inside microbore column. This monolith Allows rapid separation of common proteins, Also we successfully preparedanion exchange monoliths poly (GMA-co-EDMA) modified with Diethylamine inside 1.0 mm id and 0.05mm. id columns for the separation of DNA samples, Methylated DNA, and SNP, Monoliths have great potential as highly efficient catalyst support. For this purpose, trypsin is then Introduced to the monolithic support for high speed protein digestions. As catalyst support, monolith Also facilitates the flow of organic synthesis. Poly (GMA-co-EDMA) -based Monoliths modified with ligands of 5-amino-1,10-Phenanthroline and chemical immobilization of Pd was used for flow-through Suzuki-Miyaura cross-coupling reaction, Enrichment of phosphopeptides from the digest of the protein mixture is conducted using immobilized metal ion (Ti4 +) affinity chromatography (IMAC) monolith.**

Keywords*— *Organic Polymer Monolith, synthesis, bioanalitycal.

I. INTRODUCTION

Monolith is a continuous porous material that consists of two main pore types, namely macropores (flow-through pores) which causes convective flow in the mobile phase, and mesopores (diffusion pores) that provides a high surface area forinteraction between the analyte to the stationary phase. There are also a small number of micropores (Urban et al., 2008; Nema et al., 2013). Macropore volume fraction of porosity external show while mesopore show internal porosity (Al-Bokari et al., 2002). According to the IUPAC classification, micropore is a pore with a diameter smaller than 2 nm, having a diameter of 2-50 nm mesopore, and macropore greater than 50 nm (Urban et al., 2008; Svec, 2010). Illustration monolith columns and layout of pores in the monolith is shown in Figure 1.

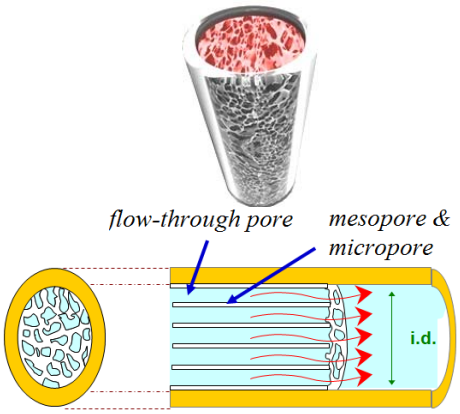

Fig 1. Illustration monolith

Advantages column monolith rather than a column of particles mainly lies in the ease of production can be made directly in the column by in-situ and covalently bound to the walls of the column, so it does not require a stopper (frit) to hold the monolith in place (Ueki et al., 2004 ; Hilder et al., 2004; Nema et al., 2013). While the column of particles, frit were placed at the end of the column to hold the particles remain in the column also affect the performance of the column. Ideally frit must be porous enough to allow the mobile phase flow uniformly along the column. Besides the problem of bubble formation and reaction between the analyte with frit material is also noteworthy. These things if not addressed properly can cause particle columns to be inefficient.

Another advantage of monoliths is the selectivity of the monolith can be easily modified in-situ with the addition of active group which selectively through copolymerization of monomer functional, post-polymerization for modification of reactive sites, as well as a photo-grafting on the surface of the monolith (Tian et al., 2008; Schaller et al., 2006). Monolith also has a higher porosity than conventional column particles, causing a greater permeability and low back pressure (Svec et al., 1995). Therefore the monolith can be applied to high flow rates at

low pressure without reducing the efficiency of the separation and no potential to cause damage to both the instrument and the column. Analysis can be performed in a shorter period of time (Vlakh et al., 2009; Arrua et al., 2012).

In contrast to the flow of eluent in the column flow diffusion particles, a monolith column eluent flow in convection, is shown in Figure 2. (Moyna, 2012). In the column directed stream surrounds a majority of particles around the particles and separation depends on the diffusion of analyte mass transfer in the pores of the particles, which this diffusion process occurs very slowly and less suitable for large-sized molecules. While the monolith column eluent is forced to flow through porous media monolith. Permeability and connectivity to the structure of the monolith ensure constant interaction between the mobile phase and stationary phase even at a flow rate that is high though, led to an increase in the mass transfer and lower the peak broadening due to eddy diffusion (Hilder et al., 2004; Vlakh et al., 2009; Svec et al, 2003).

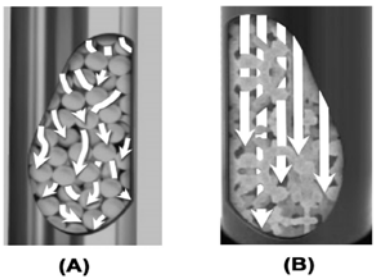

Fig 2. The flow of the eluent on a column of particles (A) and the monolith column (B)

Broadly speaking there are two types of the monolith that is silica and an organic polymer. Comparative morphology of the organic polymer-based monolith with silica based monolith is shown in Figure 3. Monolith silica prepared by a multi-step process of sol-gel more easily controlled to produce a regular pore structure and uniform and able to provide the separation process with high efficiency, especially for separation a small molecule. Monolith of this type have a better mechanical stability and relatively experienced swelling in the solvent compared to the organic polymer monolith. However, silica based monolith has a low pH stability (can only be used at pH 2-8). At a pH higher than 8 silica will be degraded, causing poor efficiency and reproducibility and high back pressure. While at a pH of less than 2, the monolith silica silylether bond will be hydrolyzed causing loss of ligand bound. Silica monolith also degraded rapidly in some phase of motion, such as phosphate buffer. Besides, the silanol effect often causes irreversible adsorption that can cause serious problems, especially in the analysis of biological samples / biomolecules including DNA. Silica-based monolith preparation process is usually multi-step making it more complicated than the preparation of organic polymer-based monolith (Ueki et al., 2004; Schaller et al., 2006; Umemura et al., 2006; Nema et al., 2013). silylether bond on silica monolith will be hydrolyzed causing loss of ligand bound. Silica monolith also degraded rapidly in some phase of motion, such as phosphate buffer. Besides, the silanol effect often causes irreversible adsorption that can cause serious problems, especially in the analysis of biological samples / biomolecules including DNA. Silica-based monolith preparation process is usually multi-step making it more complicated than the preparation of organic polymer-based monolith (Ueki et al., 2004; Schaller et al., 2006; Umemura et al., 2006; Nema et al., 2013). Besides, the silanol effect often causes irreversible adsorption that can cause serious problems, especially in the analysis of biological samples / biomolecules including DNA. Silica-based monolith preparation process is usually multi-step making it more complicated than the preparation of organic polymer-based monolith (Ueki et al., 2004; Schaller et al., 2006; Umemura et al., 2006; Nema et al., 2013). Besides, the silanol effect often causes irreversible adsorption that can cause serious problems, especially in the analysis of biological samples / biomolecules including DNA. Silica-based monolith preparation process is usually multi-step making it more complicated than the preparation of organic polymer-based monolith (Ueki et al., 2004; Schaller et al., 2006; Umemura et al., 2006; Nema et al., 2013).

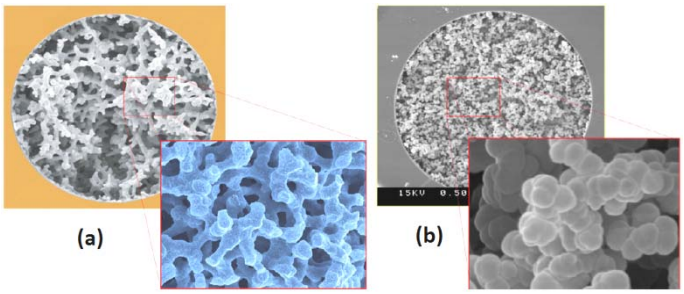

Fig 3. The difference in the morphology of the silica-based monoliths (a), an organic polymer monoliths (b)

Organic polymer-based monolith little more vulnerable than the silica-based monolith in terms of mechanical strength is relatively low because of the likelihood of swelling and shrinking when it comes in contact with some organic solvents. The other drawback is the difficulty in controlling the size of the flow-through pores that are generally not uniform and the presence of micropores which lead to the spread of the analyte causing peak broadening and ultimately reduce the efficiency of the separation of small molecules. However, the organic polymer monolith has the advantage of stability in a wide pH range (pH 2-12) and high temperature, inert to biomolecules, as well as the flexibility and versatility, especially in the case of post-modification for more diverse applications. Monolith organic polymers can be prepared easily (single-step),

Weakness in the organic polymer monolith can be overcome by optimizing the composition of the functional

monomer, crosslinker, porogen, temperature and polymerization time period is used so as to produce the pore distribution in accordance with the desired application and good mechanical strength (Svec, 2010). Besides these flaws, the organic polymer monolith has an area per volume that is lower than the porous particle media, so that in some applications the capacity per cycle is limited. However, this is compensated by the high flow rate, so that the productivity per unit of time to be as good or even better than the media particles (Vlakh et al., 2009; Svec et al, 2003).

Good monolith organic or silica-based polymers are synthesized directly by means of insitu polymerization in a stainless steel column, or fused silica capillary which serves as a template (mold) (Schaller et al., 2006; Svec et al, 2003). Monolith organic polymers can be synthesized by thermal polymerization radical, UV radiation, atapun gamma-ray radiation from a mixture comprising monomers, free-radical initiator and porogen, while silica monolith synthesized via sol-gel process by hydrolysis and polycondensation of alkoxysilanes (Hilder et al ., 2004; Arrua et al., 2012).

II. Preparation of Organic Polymer Monolith

Making the monolith can be done with a variety of functional monomers election, porogen, and polymerization conditions depending on the desired application. Variations on these components will lead to significant changes in the pore size distribution, surface area, surface chemistry properties, the degree of rigidity, swelling and shrinking (Svec et al., 2003; Hilder et al., 2004).

Monolith organic polymers can be prepared by various methods such as radical polymerization with the induction of thermal (thermal polymerization) as well as UV and LED (photopolymerization), the polycondensation reaction and ring opening polymerization metathesis depends on the type of stationary phase to be made (Nema et al., 2013; Arrua et al., 2012). Photopolymerization have advantages compared to thermal polymerization, which is faster and can be completed in a matter of minutes at room temperature compared to thermal polymerization which takes several hours. Because photopolymerization can be done at room temperature, then some kind of porogen with a low boiling point such as methanol, ethanol, chloroform, ethyl acetate and hexane can be used. In addition to photopolymerization method capable of placing the polymer in a certain part desired by covering other parts of the UV exposure. However, this method requires a monomer that is transparent to UV and UV transparent mold with a small size in one dimension. Because of these limitations cause photopolymerization method is only used for special applications and less popular than by thermal polymerization which has become a standard method in the manufacture of monoliths (Svec, 2010).

Organic monolith made of a mixture consisting of functional monomers and crosslinking monomers polymerized in the presence of an initiator and porogen. Schemes manufacture organic polymer monolith radical polymerization is illustrated in Figure 4. Dissolved oxygen must be removed from the monomer mixture by flowing nitrogen or sonication. Because of the oxygen trap free radicals in solution, causing the polymerization rate becomes slow. The mixture of monomers, crosslinker, porogen and initiator then included in the column. Polymerization begins when the mixture is heated (thermal polymerization) or initiated by UV (photopolymerization) depends on the initiator used, to form free radicals and maintain radical polymerization process over a given period.

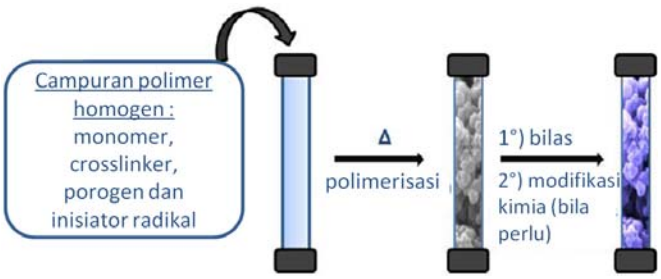

Fig 4. Schematic manufacture organic polymer monolith

Polymerization monolith is a chain polymerization (chain polymerization), initiated by free radicals and occurs in three stages: initiation, propagation and termination. The initiator generates a high-energy radicals immediately react with monomer and converting it into a new radical species which will react with other monomers. This happens on an ongoing basis in line with the growth of the polymer chain. During polymerization takes place, formed the core polymer gel-like insoluble porogen. This polymer core will grow larger and join together to form clusters up to then meet with other clusters to form a porous network structure. The pore size and nature of the monolith are formed depending on the type and concentration of monomers, crosslinker, porogen,

III. Organic Polymer Surface Modification Monolith

The chemical properties of the surface of the organic polymer monolith can be arranged by direct copolymerization using functional monomers with the specific properties of the polymer mixture. It is quite easy to do because the manufacture of the monolith can be done in just one step, and the variety of types of monomer and crosslinker carrier specific functional groups are available commercially. But the need to be optimized polymer mixture back to each addition of a new monomer. The functional group obtained in this way is also not completely lie on the surface. A number of functional groups to be buried in the polymer matrix and can not be accessed by the analyte so that less effective and efficient.

Another way to set the monolith surface chemical properties, namely the post-modification approach, in which the active group on a monolith modified by treatment with certain chemical compounds to generate functional groups appropriate desired properties. From the same polymer monolith can be made a wide variety of surface properties according to the desired application. Although this method consists of two phases, but phase this modification allows the optimization of surface chemical properties and porosity of the monolith is carried out separately. Polymer blends only need to be optimized once and does not need to be optimized back when you want to change the surface properties of the monolith. In addition to the functional groups obtained through the approach of post-modification

lies entirely on the surface so that it becomes more effective and efficient both in terms of material usage and interaction chromatography with the analyte (Nordborg and Hilder, 2009; Svec, 2010; Sabarudin et al., 2012). The number of active groups can be controlled by adjusting the time and temperature during the process of modification reaction takes place (Ueki et al., 2004).

Another way to modify the surface of the monolith is by grafting polymer chains on the surface of the polymer monoliths, which can be done by a chemical reaction and polymerization with UV initiation. This method makes it possible to add some group functions together on a surface of the monolith. However, the efficiency of the grafting method is still not very satisfactory, and there may be swelling in the grafting polymers which cause a decrease in the permeability of the monolith (Li et al., 2009; Arrua et al., 2012; Sabarudin et al., 2012).

IV. Organic Polymer Monolith Column Applications For bioanalytical

Monolith technology and its application is growing rapidly in the last decade and even now play a very powerful role and importance in the science of separation (separation science) as well as other chemical fieldfor the purpose of rapid and accurate analysis in research related to genomics, proteomics, Metabolomics, bio-elementomik, micro reactor for the synthesis of nutritious ingredients, solid phase extraction, peptide mapping, and wide-genome analysis, catalyst support.

Researcher (Sabarudin et al 2011-2018) Already have experience mensistesis various organic polymer monoliths for bioanalytical applications in Busang. We create a monolithic microbore anion exchange column which is poly (glycidyl methacrylate-CO ethylene dimethacrylate) Or (poly- (GMA-co-EDMA) with porogen consisting of 1-propanol, 1,4-butanediolAnd water are synthesized in situ copolymerization in silicosteel tubing (1 mm id, length 100 mm) for the separation of ssDNA and dsDNA, Monolith HPLC columns that are used as it was able to demonstrate the separation of 20 bp DNA ladder, a 100 bp DNA ladder, and pBR322 Hae III digest. Generally methods capillary electrophoresis (CE) is often used for the separation of these samples, but still a challenge for the CE to separate the DNA fragments of 123 bp and 124 bp in pBR322 Hae III digest. It is very impressive from a monolith made by Sabarudin et al, in addition to being able to separate the various size fragments of DNA (ssDNA and dsDNA), also in its ability to separate accurately the DNA fragment of 123 bp and 124 bp, which until now still can not be separated completely with CE , Furthermore, oligonucleotides(DT12 - dT18)can be separated efficiently in just 5 minutes. Perhaps this separation timeHampir together with using a reverse phase (RP) -HPLC monolith (Lubbad et al, 2002), but monolith made Sabarudin et al have a great advantage in the application, which did not use the ion pairing reagent and solvent organik.

Monolith for DNA separation was developed further to be able to separate the methylated DNA and single nucleotide polymorphism (SNP) that is very useful for early detection of disease. For this purpose, we conduct further optimization of themathacrylate-based monolith through changes in the column housing the place of in situ copolymerization monolith to reduce the size of the inner diameter (id) of 1 mm id to 0.5 mm id This is intended to reduce the influence of unequal heating along the diameter of the column housing and to suppress the increase in gravitational settling effect so that the resulting monolith more homogeneous. Then do too optimization more detail on composition of total monomer (% T) and crosslinker (% C) and the ratio of tertianary porogen solvents to produce a monolith with a porosity of optimum in terms of balance between the proportion of flow-through pores (for mass transfer in the convective) and a narrow mesopore size distribution so that it will be able to separate and detect the test samples for DNA methylated (with a difference of only one methyl) and SNP. less than 6 minutes), Using a linear gradient elution mode, REsolusi (Rs) for samples of oligo (dT) 12-18 ranges between 1,76 to 5,00, showing excellent separation (baseline resolved). This result is better than the monolith produced by us earlier (Sabarudin et al. 2012), where some peaks still have a resolution of less than 1,5 so that the separation and detection is still less than perfect. Similar results were shown with the separation of 50 bp DNA ladder which contain the 16 fragments of DNA, which fragments can be separated and detected well. T monolith columns with% 40 and% C 25 and then applied to a methylated DNA sample consisting of three fragments, namely the methylated DNA that do not contain methyl, contain 1 methyl, and contains two methyl groups. The principle of separation of methylated DNA withweak anion exchange this monolithwere generally similar to the principle of separation of oligo (dT) and DNA ladder, ie differences in the strength of interaction between the negative charges on the DNA with a positive charge on the stationary phase. However, in the application of this methylated DNA, the third sample length and sequence DNA has the same sequence, so that the difference is the amount of methyl contained in DNA. Methyl their will hinder interaction between the negatively charged phosphate groups with positively charged groups on the stationary phase, so more methyl contained would lead to interaction with the stationary phase is getting weaker. DNA containing many methyl will elute first from the column. calculation resolution (Rs) show that separation of methylated DNA in the range of 1:50 to 1:52. These results demonstrate the ability of the monolith column in separating methylated DNA sample is very ideal (baseline resolved) and in a relatively short time, ie less than 10 minutes.Monolith is also capable of separating the single nucleotide polymorphism (SNP), with a peak detection clearly split of 4 samples SNPs used in this study. Yet still require increased resolution for the separation of these SNPs.

We (Sabarudin et al, 2013-2018) also has develop monolith is not limited to media separationbut also to media selective enzymatic reaction and concentration of biomolecules. For this purpose, we make Immobilized Metal-ion Affinity Chromatography (IMAC) organic polymer-based monolith which has a selective affinity to biomolecules that plays an important role for the collection and enrichment of biomolecules such as proteins, DNA, and oligonucleotides. in the study this, Has made three types Monoliths namely (a) Lauryl methacrylate / LMA C12-Monolith for identification / separation phosphopeptida, (b) trypsin-immobilized monolith for on-line digestion of

proteins into peptides, and (c) Ti^{4+} -Immobilized monolith to concentration Phosphopeptide selectively.

Lauryl methacrylate / LMA C12-Monolith has tested its performance for the separation of alkyl benzene homologues and able to provide excellent separation of the mixture uracyl, toluene, ethylbenzene, propylbenzene, butylbenzene, and amylbenzene. LM ore far more detailed test is carried out using isocratic mode and gradient on a variety of conditions to achieve optimum separation efficiency. Further performance of the monolith are able to separate the 10 proteins are aprotinin, ribonuclease A, insulin, cytochrome c, trypsin, transferrin, conalbumin, myoglobin, β-amylase, ovalbumin within 2 minutes.

Trypsin-immobilized monolith microreactor (TEAMM) For on-line digestion of protein into peptide-peptide has been successfully created and evaluated for on-line digestion of cytochrome c and beta-casein. In this study, microreactor nanobiokatalis mentioned made using nanopori monolithic polymer (poly- (GMA-co-EDMA)) in a column silicosteel in-situ copolymerization between monomers glycidyl methacrylate (GMA) and ethylene glycol dimethacrylate (EDMA). Radical initiators (AIBN) is used in the process in the presence of porogen 1-propanol / 1,4-butanediol / water. Further, the immobilized trypsin into the monolith. The process will produce a microreactor nanobiokatalis which in this study is called trypsin immobilized monolith microreactor (Timm), as a comparison carried out also making microreactor nanobiokatalis the same and are activated by the addition of gluteraldehyde as crosslinker, so-called trypsin immobilized monolith microreactor-glutaraldehyde (Timm-G). The addition of gluteraldehyde is expected to increase the stability of enzymes in the monolith in terms of the formation of a multilayer coating of enzymes, so the effectiveness of the process of digestion sample of peptide-peptide proteins become increasingly high. Testing of total surface area and pore properties monolith performed using Brauneur-Emmett-Teller (BET) and Inverse Size Exclusion Chromatography (ISEC). Permeability and Timm Timm-Gnextsystematically tested. Characterization of surface morphology and Timm Timm-G was performed using instruments Scanning Electron Microscopy (SEM-EDX), the identification number of the nitrogen atom-G Timm Timm and carried through the approach to the atomic percent relative to the EDX.Timm that have formed chemically modified by the immobilization of trypsin stabilized in Benzamidine hydrochloride and carbonate buffer. In this immobilization process occurs epoxide ring opening reaction of the polymer monolith to trypsin. Some of the test parameters used in this research to produce a microreactor in its optimum condition that the time variation of immobilization, the effect of adding glutaraldehyde in column trypsin monolith, the variation of the residence time (residence time) a sample of the process of digestion, and variations in the column temperature trypsin monolith in the process of digestion protein sample. Based on the results of mesoporous and macropore proportion ISEC (flow-through pore) balanced ie 65.85%, and 28.20%. Results of SEM-EDX showed the highest nitrogen content that represents the number of immobilized trypsin in the monolith by 12, 40% is produced from trypsin monolith with immobilization time of 4 hours without the addition of glutaraldehyde. Nanobiokatalis microreactor at optimum conditions are used for sample digestion process protein (β-casein and cytochrome C) quickly and efficiently. TEAMMThese proteins capable mendigest in a short time (1-50 minutes, depending on the type of protein) and accurate. From the test results using cytochrome c and beta-casein, keakuratanya mencpai 100% for men-digest the protein into peptide-peptide. Another advantage of TIMM made in this study is not the case so that the leaching of trypsin TEAMMcan be used over and less because it has a high degree of stability. This is because trypsin is chemically immobilized by covalent bonds on poly- (GMA-co-EDMA) monolith. This character is not owned by a variety of research on enzyme immobilization (enzyme immobilization) and the development of enzyme column that has been previously published.

Ti^{4+} -Immobilized monolith also has been created for the concentration fosfopeptida,Fosfopeptida present in a large number of biological mechanisms. Fosfopeptida plays an important role in gene regulation, signal transduction and metabolic control in eukaryotic cells. Abnormail phosphorylation can cause various diseases, including cancer. However, the existence of fosfoprotein very low, ranging from 1-2%. In this study, manonolith nanopori developed for the separation and detection of fosfopeptida in proteomic analysis. Nanopori column-based monolith made in silicosteel column (100 x 1.02 mm id) through in-situ copolymerization reaction using glycidyl methacrylate and ethylene dimethacrylate. Immobilized Ti4 + ion on the monolith after post-modification using aminomethyl phosphonic acid (AMPA). Physical character of columns monoliths, such as morphology, elemental analysis, surface area analysis, permeability and pore distribution is characterized more detail. The result of optimization of the monolith has a good mechanical stability and high permeability, as well as having the ideal proportions of macropores, mesoporous and microporous amounted to 27.50%; 66.57% and 5.93% respectively. Monolith nanopori -IMAC Ti4 + are further applied to the separation and detection of sample digestion fosfopeptida protein (β-casein and cytochrome-c) and tyrosine phosphorylated peptides. The results obtained showed that nanopori Ti4 + -IMAC based monolith provides high selectivity and efficient for detection fosfopeptida mesoporous and microporous amounted to 27.50%; 66.57% and 5.93% respectively. Monolith nanopori -IMAC Ti4 + are further applied to the separation and detection of sample digestion fosfopeptida protein (β-casein and cytochrome-c) and tyrosine phosphorylated peptides. The results obtained showed that nanopori Ti4 + -IMAC based monolith provides high selectivity and efficient for detection fosfopeptida mesoporous and microporous amounted to 27.50%; 66.57% and 5.93% respectively. Monolith nanopori -IMAC Ti4 + are further applied to the separation and detection of sample digestion fosfopeptida protein (β-casein and cytochrome-c) and tyrosine phosphorylated peptides. The results obtained showed that nanopori Ti4 + -IMAC based monolith provides high selectivity and efficient for detection fosfopeptida in a complex biological sample.

Furthermore, we also developed a microreactor-based monolith (catalytic monolith) for cross-coupling reaction of Suzuki-Miyaura. With this monolith,

reactionsorganik for the manufacture of the drug substance only takes in seconds to minutes.

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For more details, we will discuss further in a seminar later on September 22, 2018, State University of Surabaya.

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