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Membrane support formulation and carrier selection in supported liquid membrane for extraction of zwitterionic form of Glutamic acid

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ABSTRACT

Glutamic acid (GA) is an amino acid which is usually appear in the zwitterionic form in solutions. At present, cation and anion exchange carriers are widely use to extract this amino acids. However, the carriers only function either at high pH or low pH. Although, the pH can be adjusted by adding certain chemical to reach acidic or basic region, but it may lead to additional byproducts formation and affect the extraction process. In the current study, supported liquid membrane (SLM) was used to extract the zwitterionic form of GA from the aqueous solution without any pH adjustment. In the SLM process, the determination of the best carrier in liquid membrane formulation is important for achieving high extraction of GA. Hence, different types of carriers such as trioctylamine (TOA), tridodecylamin (TDA), tri-n-octyl phosphine oxide (TOPO), mixture of 50% TOA and 50% TDA, aliquat 336 and di-2-ethylhexyl phosphoric acid (D2EHPA) in 2-ethyl-1-hexanol were investigated. The polyethersulfone (PES) membrane with and without graphene membrane supports were prepared, characterized and used as the support in the SLM. The incorporation of graphene in PES membrane had increased the surface contact angle and tensile stress from 80.96 ± 1.92° to 97.8 ± 1.46° and 650.684 kPa to 1079.59 kPa, respectively. Aliquat 336 was identified as the best carrier with 93% of GA extraction.

1. Introduction

GA is an amino acid which consists of two carboxyl groups and an amino group. GA was discovered and identified in 1866, by the German chemist Karl Heinrich Ritthausen [1]. GA is used as flavor and taste enhancer, neural drugs for nerve stimulant, animal feeds for the growth of poultry and precursors for synthesis of various kinds of specialty chemicals [2,3]. GA and its valuable derivatives are growing demand in various application. The global GA market is expected to grow due to rising application in food additives, pharmaceutical and animal food. The global GA market is estimated to reach >4 million tons by 2023, growing at a CAGR of above 7.5% during 2014 to 2023 [4].

GA is produced by hydrolysis of plant proteins like wheat gluten or soy bean protein [5]. During hydrolysis, glutamine in proteins converted to GA [6]. Nowadays, production of GA by fermentation is getting attention due to the increasing environmental constraints. Corynebacterium species are commonly used for economic production of GA through fermentation [7]. The main challenge of biorefinery in fermentation-based routes is the technical difficulties associated with costly recovery of desired product [8].

Nowadays, supported liquid membrane (SLM) process is getting more attention for selective separation of heavy metals and chemicals. SLM is a simple low cost process which is able to promote highly selective separation with a small amount of organic phase with carrier [9]. Furthermore, SLM process shows a great potential to extract and recover the desired solute in a single step. This process provides maximum driving force for the separation of desired

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product, thus leads to high extraction rates [10]. In SLM, polymeric membrane support plays key role in the stability and performance of the process. For immobilization of liquid membrane, a microporous polymeric membrane with high hydrophobicity, high porosity, small pore size and proper tortuosity should be used [11]. The membrane support also should be chemically stable on exposure to feed, stripping and impregnating solvents.

Recently, mixed matrix membranes (MMMs) have become a prominent area of current research and development [12]. It is a type of membrane formed by incorporating fillers in polymeric matrix. The additional of fillers in polymeric membrane improves the membrane separation performances and properties of the membrane such as stiffness, toughness, chemical stability, electrical conductivity, and resistance [13]. Graphene is a super strong inorganic material that possess high thermal conductivity and mechanical properties [14]. It can be mix with other materials to make strong and tougher composite materials. It has been stated that, the hybrid polyethersulfone (PES)-graphene membrane support is suitable to be used in SLM for acetic acid removal [15].

Apart from that, GA can exists as a free form or in a salt form in the fermentation broth depending on the pH. GA has low solubility at its isoelectric point (pI) [16]. The pI of the GA is 3.22 [17]. When pH < pI, the GA will be more acidic and in the anion form. At pH > pI, it becoming more basic and in the cation form [5]. At pI point, the GA is in the zwitterionic form and carry no net charge [18]. Both amine and a carboxylic group in the GA are charged and form a strong intramolecular bond at the pI point. Even, the liquid anion or cation exchangers also not strong enough to break this bond. In consequence, they failed to extract the amino acid from aqueous phase [19]. The extraction of amino acid by liquid cation exchangers or liquid anion exchangers works only either at high pH or low pH [19]. However, the additional of chemicals to shift the pH to an acidic or basic range may lead to additional byproducts and effect the extraction process.

In present study, the extraction GA at their pI using SLM was studied without adjusting pH value of solution. Basically, the carrier or extractant in SLM is choose based on selectivity of the components present in the feed phase [11]. It have been reported that the carboxyl group can be extracted by solvation with carbon-bonded oxygen-bearing extractants, solvation with phosphorous-bonded oxygen bearing extractants and extraction by proton transfer or by ion-pair formation, with high molecular weight aliphatic amines and their salts [20]. Furthermore, carboxylic acids also can be extracted by using ionic extractants such as Aliquat® 336 [20]. Therefore, the main aim of this study to determine which carrier is the best for the GA extraction. In addition, custom made flat sheet membrane support based on polyethersulfone (PES) with and without graphene were prepared and characterized for the potential used as the membrane support in the SLM process.

2. Materials and methods

2.1. Materials

Polyethersulfone (Radel® A, Solvay, USA), dimethylacetamide, DMaC (Sigma Aldrich), polyethylene glycol, PEG 200 (Sigma Aldrich), and graphene nanopowder with particle size around 25 μm (Low Dimensional Materials Research Centre, Universiti Malaya, Malaysia) are the materials used in dope solution for membrane fabrication. Tap water was used as a coagulation medium. The liquid membranes were formulated using different carriers of TOA (Sigma Aldrich), TDA (Merck), TOPO (Sigma Aldrich) and Aliquat 336 (Merck) dissolved in 2-Ethyl-1-hexanol (Sigma Aldrich) diluent. Sodium hydroxide, NaOH (Merck) and GA (Acros/Belgium) were selected as the strip and feed phase, respectively. The porosity test for membrane was conducted by using olive oil purchased from Delima Oil Products Sdn Bhd.

2.2. Membrane fabrication

A PES and a hybrid PES-graphene flat sheet membrane supports were fabricated at casting thickness of 400 μm by using a dope solution (42.5 wt% of PEG 200, 15 wt% of PES, 42.5% of DMaC without graphene and with graphene (0.1 wt% of graphene nanopowder (%w/w of polymer)) respectively through VIPS technique at environmental humidity of 86 ± 2%. The details about the methods for the membrane fabrication has been reported in previous publication [15].

2.3. Membrane characterization

2.3.1. Membrane morphology

Nitrogen liquid were used to fracture the membranes. The fractured membranes were sputtered with platinum and morphology of the membrane were observed by using Field Emission Scanning Electron Microscopy (FESEM) model JEOL JSM-5410LV, Japan.

2.3.2. Membrane hydrophobicity

The surface hydrophobicity of the membranes were determined by using optical contact angle measurement system (CAM 101 optical Contact Angle Meter, KSV Instruments). A micro syringe was used to drop 5 μl of water on the membranes surface. The average contact angle of membranes from three different membrane locations were calculated.

2.3.3. Porosity measurement

The 10.5 cm × 4 cm membrane supports were dried in a vacuum oven at 80 °C for 24 h and weighed it as W_1. Latter, the membranes were soaked in olive oil for 24 h. The excess olive oil on the membrane surfaces were removed by using filter paper and weighed as W_2. The average porosity of two membrane samples were reported. The membrane porosity, ε (%) were calculated using the Eq. (1).

$$\varepsilon = \frac{W_2 - W_1}{\rho V_1} \times 100 \quad (1)$$

where W_1 and W_2 is the weight of the dry and wet membrane in g respectively. V_1 is volume of the membrane (cm^3) and p is the density of olive oil (0.8 g/cm^3). The details about the methods for the porosity measurement has been reported in previous publication [21].

2.3.4. Mechanical strength

Tensile stress (kPa) of the two rectangular shaped membrane samples (5 cm × 2 cm) from pristine PES and hybrid PES-graphene were measured by using a universal testing machine Shimadzu EZ-LX at a loading velocity of 5 mm/min and the average tensile stress of each types of membranes were calculated.

2.4. Supported liquid membrane

The prepared hybrid PES-graphene flat sheet membrane support was immersed in different individual organic liquid membrane solutions as TOA, TDA, mixture of 50% TOA and 50% TDA, TOPO and Aliquat 336 in 2-Ethyl-1-hexanol for 24 h. The concentration of the carrier was fixed at 0.5 M. The excess liquid membrane on the membrane surface was cleared by using filter paper. The SLM was fixed between two Teflon compartment membrane cells and attached to the SLM system. 10 g/L GA solution and 0.5 M NaOH solution were pumped in counter-current flow into

the feed and strip phase of membrane cell respectively with flow-rate of 50 ml/min. The samples were taken from the feed side for every 2 h for a period of 8 h.

2.5. Glutamic acid extraction yield

Eq. (2) was used to calculate the GA extraction yield during the SLM process:

$$Extraction\ yield(\%) = \frac{[GA]_{fi} - [GA]_{fo}}{[GA]_{fi}} \times 100 \quad (2)$$

where $[GA]_{fi}$, $[GA]_{fo}$ are the initial and final concentration of GA at feed phase respectively. Synergy Hydro C18 HPLC column (Phenomenex, 250 mm × 4.6 mm, 4 µm particle size) that attached to the Agilent HPLC 1200 was used to determine the concentration of GA. GA was detected by using 0.02 M potassium dihydrogen phosphate (pH 2.9) as mobile phase with ultraviolet (UV) detector at 221 nm wavelength.

3. Results and discussion

3.1. Membrane characterization

3.1.1. Membrane morphology

Based on Fig. 1(a), a symmetric cylindrical microvoids structure was formed across the pristine PES membrane. However in the hybrid PES-graphene membrane, very thin dense sublayer formed at the top of the membrane followed by an asymmetric cylindrical microvoids as shown in Fig. 1(b). The addition of graphene into PES improved the hydrophobicity of the membrane and reduce the interaction of water towards PES-graphene film. Hence, the diffusion rate of water into casting solution became lower and the solvent diffusion rate from the surface casting is became higher. Thus, this lead to a dense sublayer on the top of the membrane [22]. Furthermore, larger pores were formed at the bottom part of the hybrid PES-graphene membrane due to the fusion of two polymer-poor phases before wall solidification in the membrane.

3.1.2. Membrane hydrophobicity

Fig. 2 shows the contact angle results of the pristine PES and hybrid PES-graphene membranes. Theoretically, the membrane is considered as hydrophobic and super hydrophobic membrane if the contact angle value > 90° and >150°, respectively [15]. Based on the results, contact angle of the pristine PES membrane is

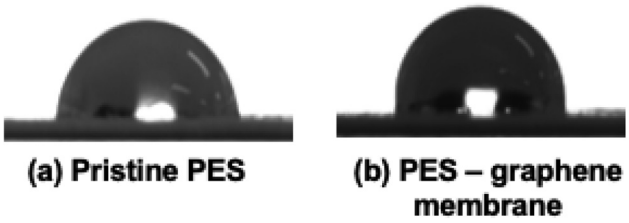

Fig. 2. Contact angle of the (a) pristine PES and (b) hybrid PES-graphene membrane.

80.96° ± 1.92. Hence, it is a hydrophilic membrane. Addition of the hydrophobic graphene in PES membrane had increased the contact angle to 97.8° ± 1.46 and improved the hydrophobicity property of the membrane support. Basically, the hydrophobic membrane support can promotes capillary force which helps retaining the liquid membrane within the pores of the support [15]. This will prevent the liquid membrane leakage and enhanced the stability of the SLM operation. Therefore, the hybrid PES-graphene membrane was chosen as the membrane support in this SLM for GA extraction study.

3.1.3. Tensile stress

Tensile stress is the maximum amount of pressure that a material can resists before it breaks. The stress to break of the PES-graphene membrane prepared in this study is 1079.59 kPa s shown in Fig. 3. This value is higher compared to the pristine PES which had a value around 650.684 kPa. The existence of graphene within the PES matrix improved the mechanical strength of the membrane and lead the membrane support remains stable without any breakage in SLM system for long operation time [21].

3.1.4. Membrane porosity

Based on the literature review the number of pores, pore size, tortuosity and polarity are highly impacted the porosity of a membrane [21]. The larger the pore size, the larger the empty space inside and around the pores [21]. The average porosity of the pristine PES membrane was 66.24 ± 5.29% which is 7% greater than the hybrid PES-graphene membrane with average porosity of 59.05 ± 3.75%. Hence, the pristine PES membrane can impregnates more organic liquid membrane and extract more GA from the feed phase compared to the hybrid membrane. The hybrid PES-graphene was chosen for further experiment due to their high hydrophobicity and high mechanical strength compared to the

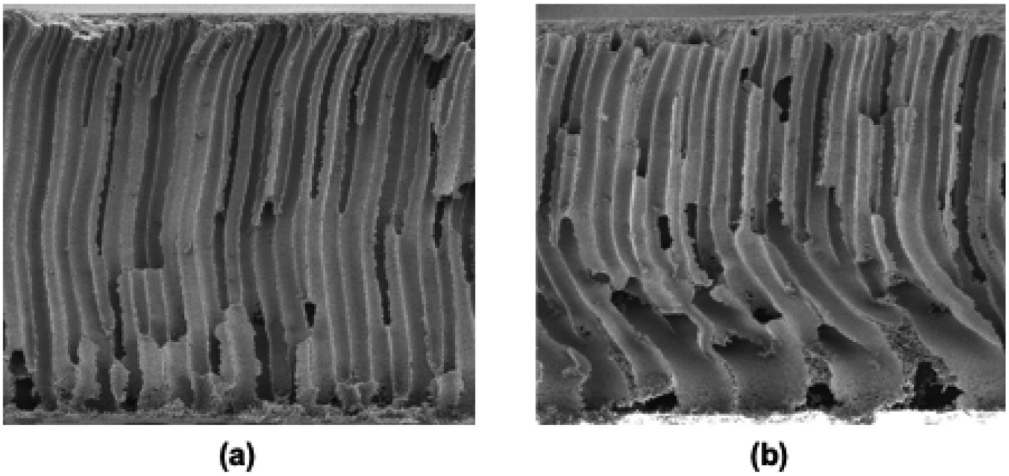

Fig. 1. Cross section of (a) pristine PES and (b) hybrid PES-graphene membrane.

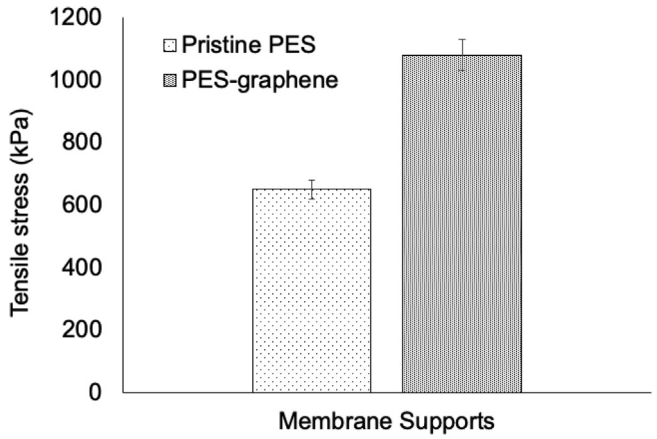

Fig. 3. Average stress to break value of pristine PES and PES-graphene membrane.

pristine PES membrane. Both properties are important in preventing liquid membrane lost from the pores of the membrane support and avoiding membrane breakage during SLM operation [23].

3.2. Extraction yield of GA

The study was conducted to find out the best carrier to extract GA from aqueous solution without any pH adjustment. In present, 10 g/L of GA was tested with different carriers (TOA, TDA, mixture of 50% TOA and 50% TDA, TOPO and Aliquat 336) at concentration of 0.5 M in 2-Ethyl-1-hexanol. 2-Ethyl-1-hexanol that used in this study is a water-immiscible diluent that has a high solvation energy and can provides higher distribution coefficient [24]. The temperature of the GA solution was kept the range of 25–45 °C to avoid α-form nucleation at low temperature of 25 °C and β-form nucleation at high temperature of 45 °C [25]. The initial pH of the GA solution was recorded at 3.25 which is near to the pI of the GA. Hence, the amine group in GA is positively charged and one of the carboxylic group are negatively charged as shown in Fig. 4 [17].

Based on the literature review, the intramolecular bond formed between zwitterion of amino acid at pI point is hard to break [19]. Therefore, the amino acid solution is usually shifted to high pH or low pH before the extraction process in order to avoid the intramolecular bond which disrupt the extraction of amino acid [19]. GA also is an amino acid, but it is special because it consists of two carboxyl groups. There are only one carboxyl group (COO^-) was charged and form an intramolecular bond with charged amine group (NH_3^+) at isoelectric point and another uncharged carboxyl group can be extracted by the carrier in organic phase as shown in Fig. 5. The carrier is interacted with the uncharged carboxyl group in GA and formed acid-carrier complex. Latter, the complex is diffused across the organic liquid membrane phase until it reached the stripping phase. Back extraction is occurred at stripping phase and the carrier is diffused back across the membrane to repeat the extraction cycle again as shown in Fig. 5 [26,27].

Based on the Fig. 6, Aliquat 336 is the best carrier with 93% of GA extraction. Aliquat 336 is a cationic extractant which can able to extract both the undissociated and dissociated forms of the acid

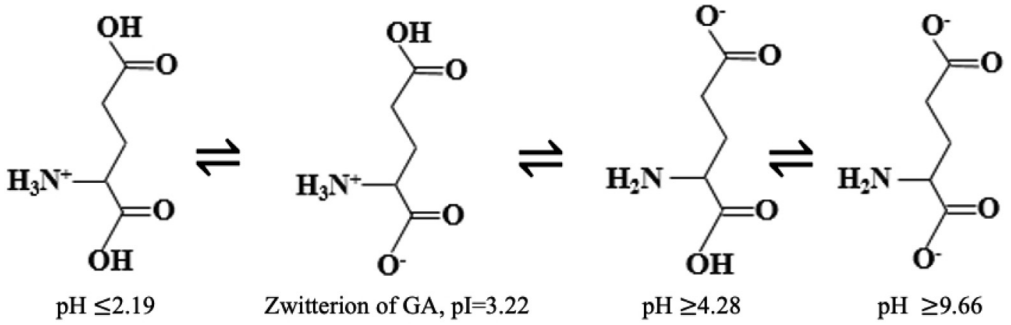

Fig. 4. GA structure at different pH.

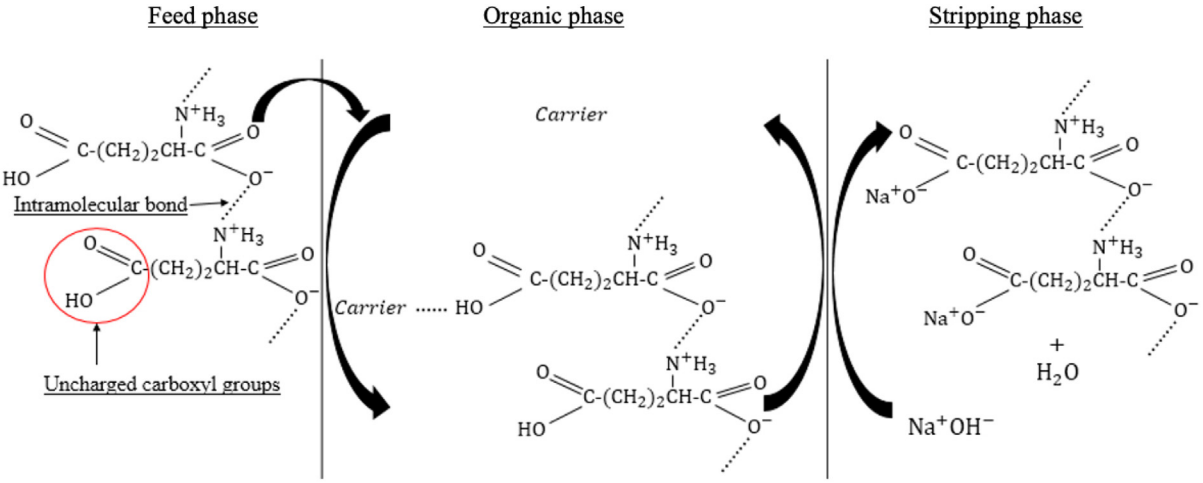

Fig. 5. Enrichment principle of GAs with carrier.

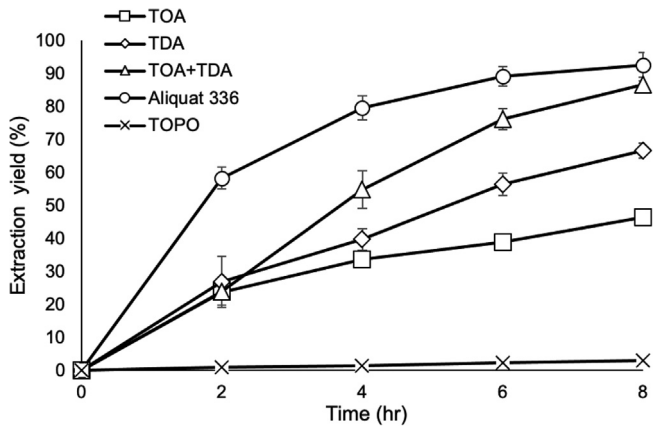

Fig. 6. Extraction yield of GA over time from aqueous solution by using 0.5 M of different carriers.

by hydrogen bonding or ion exchange mechanisms [27]. The TOPO is the least efficient carrier compare to other carriers that have been tested in this study. It extracted 3% of GA from aqueous phase. It was observed that TOPO crystal layer was form on the surface of membrane when it was exposed to air. The presence of TOPO in NaOH caused precipitation which has been reported earlier [28].

Based on the study, tertiary amines able to extract GA from the aqueous phase. The tertiary amines only can extracts the undissociate form of acids [29]. The results shows that the TDA was extracted 67% of GA. The long alkyl chains in TDA interrupt the availability of GA in organic phase for the acid–amine complexion, thus reduce the extraction efficiency. TOA was extracted 47% of GA from aqueous phase in 8 h. TOA consists of short alkyl chain and let high concentration of GA in organic phase for acid –amine complexion. The high concentration of GA in organic phase affected the transport mechanism of the complex. Therefore, the percentage of acid extracted by TOA is lower than TDA. The mixture of 50% TOA and 50% TDA was extracted more acid compare to both of the individual amines, with the extraction yield of 87%. In overall the extraction yield of GA by using tertiary amines were increasing over time. However, the efficiency of acid extraction were decreasing over time due to the decreasing concentration of GA in aqueous phase.

4. Conclusion

SLM is an effective method that can be used to separate GA from aqueous solution. The hybrid membrane support developed from the mixture of PES and graphene improved the hydrophobicity and mechanical strength of the PES membrane. Therefore, it is more preferable to be used in SLM system compared to the pristine PES membrane. Based on the study, Aliquat 336 is the best carrier for the extraction of GA at isoelectric point. It was successfully extracted almost 93% of GA from the aqueous solution without any pH shift process.

CRediT authorship contribution statement

Vikneswary Rajendaren: Investigation, Writing - original draft. **Syed M. Saufi:** Supervision, Conceptualization. **M.A.K.M. Zahari:** Supervision, Conceptualization. **Abdul Wahab Mohammad:** Resources, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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