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Extraction of Amino Acids from Their Aqueous Solutions by Using Derivatives of Polyethylene

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ABSTRACT

Sixteen sample of low density polyethylene grafted with different ratios of maleic anhydride, N-phenylmaleimid and mixture of both maleic anhydride and N-phenylmaleimid have been prepared through free radical polymerization. The grafted polymers were examined to extract several types of amino acid from their aqueous solutions. It was found that the efficiency of extraction of every amino acid was depends on the type of grafting. Maleic anhydride grafted polymer is suitable to extract the amino acid with acidity characterized, while the amino acids with basic characterized are favorable to be extracted by N-phenylmaleimid grafted polymers. Many factors affecting the efficiency of extraction have been studied ;i.e. the concentration of the amino acid solutions ,treatment time, pH of the medium ,and temperature .Regeneration efficiency was also studied by using different types of eluents according to the type of the extracted amino acid.

Key words: Amino acid, extraction, polyethylene, maleic anhydride.

INTRODUCTION

The extraction of amino acids from their sources and from the nature are always form a troublous problem to the researchers. Many methods have been employed in this field , most of these methods are too complicated and need advanced technology and many reagents .The famous methods are depended on separation by counter- current , electrophoretic and chromatographically methods [(1-2)].

In order to extract the amino acids from their aqueous solution ,different solvents have been used whereby the hydrophilic groups of amino acids can forms links with water molecules .Many different organic solvents were used to transfer the amino acids from the aqueous layer to the organic layer like octanol[(3)],normal alkanes[(4)]or cyclohexane[(5)].Stein et.al. extract amino acids with high efficiency by using ion exchanger chromatography [(6)].

Liquid membrane have been developed in extraction and separation a mixture of different amino acids[(7)].Microemulsion bulk liquid membrane was used for the selective transport ofL-tryptophan and L-tyrosine [(8)]

It was known that the chemical structure of the amino acid consist mainly of two functional groups, carboxylic acid group, which is considered as an acid functional group, and an amino group, which is considered as a basic functional group .From this assumption the amino acids are classified into three categories depending on the number of the acid and the base moieties. The three types of amino acids are acidic, basic and neutral.

This proposal was exploited in our work in order to extracts the amino acids from their aqueous solutions by using samples of polyethylene (PE) grafted with different active functional groups. Two types of functional groups are grafted to PE with different ratios, succinic anhydride as an acid function and maleimic acid as a basic function. The efficiency of extraction towards the different types of amino acids are depend on the amount and ratio of the grafted moieties on PE chain.

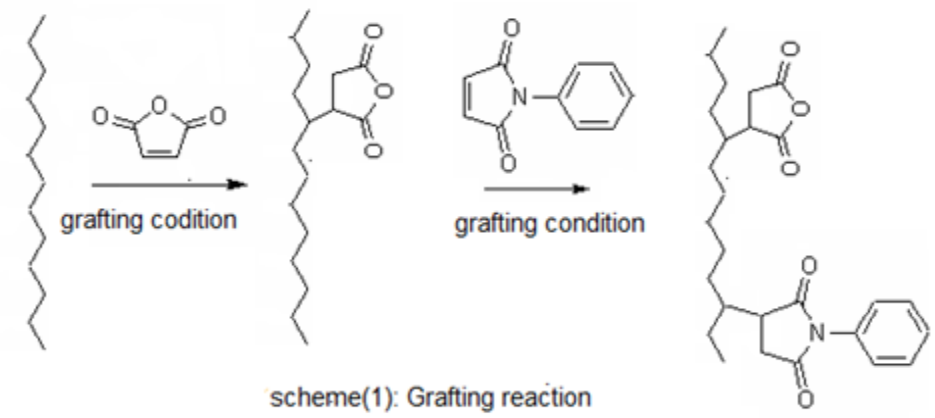
EXPERIMENTAL

Low density polyethylene (LDPE) (BDH) and the initiator Luperox- F (Donjeen) are used as a commercial products .Maleic anhydride (MAn) (Fluka) was recrystallized from dried chloroform. All the solvents, reagents and amino acids are used as received.

N-Phenyl maleimide (NPM) was prepared according to known method [9] by the reaction of MAn with equimolar of aniline in chloroform at temperature of 10°C to form the maleimic acid. The maleimic acid was converted to maleimide in the presence of acetic anhydride and sodium acetate.

Grafting reactions:
MAn grafting on to LDPE:

Two g of LDPE was dissolved in 20 ml of 1, 2- dichlorobenzene at the boiling point of the solvent. Certain amount of MAn and half amount of the initiator was added under nitrogen atmosphere (table 1). The reaction was carried out for 24hrs. . The remaining half amount of the initiator was then added and the reaction was continued for another 24 hrs. .The grafted polymer was then precipitated from acetone, filtered ,washed with acetone and dried under vacuum at 50C°.



scheme(1): Grafting reaction

Table (1): Grafting 2g of PE with different ratio of Man

Sample no.	MAn/g	Initiator / g
7	0.4	0.036
8	0.4	0.048
9	0.6	0.096
10	0.8	0.096
11	1	0.096

NPM grafting on to LDPE:

The grafting of LDPE with NPM was carried out as the grafting of MAn on to LDPE with a certain period of about 24hrs and the initiator was added as one portion (table 2).

Table (2): Grafting 2g of PE with different ratio of NPM

Sample no.	NPM in feed g/2g LDPE	Initiator g/2g LDPE
1	0.0234	0.02
2	0.117	0.02
3	0.234	0.02
4	0.468	0.02
5	0.936	0.02
6	1.17	0.02

Grafting of NPM on to LDPE grafted with MAn:

The samples of LDPE grafted with different ratios of MAn were again grafted with different ratios of NPM following the same above procedure of grafting NPM on to LDPE (table 3).

Table (3): Grafting NPM on 2g of PE grafted with different ratio of Man

Sample no.	MAn grafted PE sample	Initiator /g	NPM / g	Time /hr.
12	7	0.02	0.936	1
13	8	0.02	0.936	2
14	9	0.02	0.702	2
15	10	0.02	0.468	2
16	11	0.02	0.351	2

Evaluation of grafting degree:
MAn grafted:

The degree of grafting of MAn on to LDPE was determined by titrating the acid group with alcoholic KOH after complete hydrolysis of the grafted succinic anhydride into succinic acid by using water [10] as following:

Known weight of the grafted polymer was dissolved in boiled xylene saturated with water .The hot solution was titrated against 0.05N alcoholic KOH solution in presence of 2-3 drops of 1%thymol blue in DMF as indicator. 0.5- 1 ml excess of KOH solution was added after the color of the solution became blue, then the solution was back titrated with 0.05N HCl solution until the color changed to the yellow at the end point. The percent of maleic anhydride was calculated by using the equation:

%MAn= (N of KOH × V of KOH ×98)/ (polymer weight×5)

NPM grafted:

The degree of grafting NPM was evaluated spectrophotometrically [11] as follows:

After precipitating the polymaleamic acid from the filtrate solution that produced from grafting reaction by methanol, 0.2 ml of this solution was added to 5 ml of toluene, the absorbance of the formed complex was measured at 320 nm. The amount of the maleamic acid was calculated from the calibration curve between the absorbance and the maleamic acid concentrations. The grafted amount can be calculated from the equation:

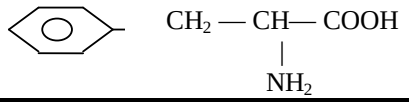
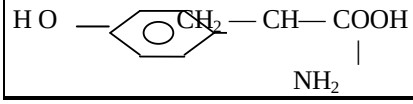
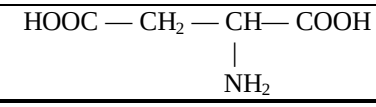
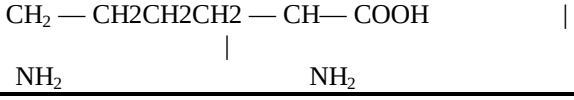
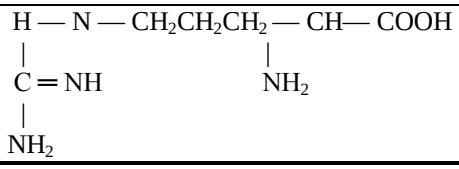
$$\% \text{ Grafting} = \frac{(C + B) - A}{m}$$

Where A represent the weight of the added maleamic acid to the reaction, B is the amount of the unreacted malemic acid, C is the weight of the polymaleamic acid and m is the weight of grafted polymer sample.

Extraction Efficiency:

Batch system technique was used in studying the extraction of amino acids from their aqueous solutions .Six different types of amino acids have been chosen in this study as shown in table (4). 0.1g of the polymeric sample was treated with 25ml amino acids aqueous solution (500ppm), the concentration of the amino acid solution was determined before and after treatments spectrophotometrically. The method depends on measuring the absorption of the complex formed between the amino acid and ninhydrine solution according to published work[12].The examined factors effecting the efficiency of extraction are ; concentration of amino acid solution , pH of the medium ,time of treatment and the temperature.

Table (4): Chemical structure of the used amino acids

Amino acid	Chemical structure	type
glycine		neutral
L-phenyl alanine		neutral
L-tyrosine		
DL-aspartic		acidic
L-lysine		basic
L-arginine		basic

Regeneration study:

The regeneration of the amino acids located on the chosen polymeric samples was studied by using different eluent solutions. In regenerate L-phenyl alanine, L- tyrosine and L-lysine hydrochloric acid was used. Phosphoric acid was used in regenerate L-arginine, while DL- aspartic and glycine were regenerate by using ammonium hydroxide solution as eluent. The concentration of eluent, time of treatment and temperature are factors studied in affecting the efficiency of regeneration process.

RESULTS AND DISCUSSION

Grafting LDPE

The reactive species (MAn and NPM) can be inserted to polyethylene chain randomly as a single functional group through their unsaturated moieties[13].The grafting reactions were carried out through free radical copolymerization process in the presence of the imitator 1,4-di(2-tet-butylperoxyjsopropylbenzene (Luperox-F) . The infrared spectrum of the grafted polymers confirms the presence of the specific functional groups related to the grafted moieties. The IR spectra of the MAn grafted PE shows two peaks at 1710cm^{-1} and 1772cm^{-1} which are the characteristic of succinic anhydride group. On the other hand the spectrum of NPM grafted PE appeared another four peaks in between (13394 – 1597) cm^{-1} and peak at 3094cm^{-1}which are related to the aromatic ring , as well as the specific two amide bands at 1697cm^{-1} and 3211cm^{-1} .

The IR spectrum of MAn –NPM grafted PE reveals the presence of the two groups grafted to PE chain. The spectrum shows two peaks at 1709cm^{-1} and at 1772cm^{-1}which are related to carbonyl groups of the cyclic anhydride and peak appeared at 1639cm^{-1} belong to the carbonyl group of the amide moiety . Also the bands of the aromatic ring appeared between (1331-1509) cm^{-1}.

The degree of grafting of MAn and NPM within the different experiments was shown in table (5,6and 7). It is clearly noticed that the degree of grafting have been influenced by the conditions of the experiments.

Table (5): NPM grafted PE

Sample no.	percent of NPM
1	0.20
2	2.46
3	4.48
4	8.94
5	21.01
6	41.76

Table (6): MAn grafted PE

Sample no,	Percent of MAn
7	10.5
8	14.27
9	25.23
10	35.74
11	41.00

Table (7): NPM and MAn grafted to PE

Sample no,	Percent of MAn	Percent of NPM
12	10.5	34.07
13	14.27	35.77
14	25.23	24.26
15	35.74	16.72
16	41.00	11.90

Extraction Efficiency:

Table (8) shows the percent of amino acids extracted from their aqueous solution by using different polymer sample. The results revealed that the efficiency of extraction depends on the chemical structure of the amino acids and the amount and type of the grafted moiety on the polymer chain. Through treatment the aqueous amino acid solutions with polymer samples, the grafted cyclic succinic anhydride moieties undergo decomposing into two carboxyl group's .Now the polymer chain contain two active site, acid from succinic acid moiety and basic from NPM moiety. Where by the acid side of the amino acid can be connected with basic grafted group side and the basic side of the amino acid connects with the grafted carboxyl group.

Table (8): Percent of extracted amino acid from their aqueous solution (500ppm) by 0.1g of different polymer samples at35^{0}C for24hrs

Polymer sample	extracted amino acid%					
	glycine	L-phenyl alanine	L-tyrosine	DL-aspartic	L-lysine	L-arginine
2	5.81	0.26	3.84	5.88	1.48	24.50
3	17.14	1.58	7.45	9.47	3.57	27
4	26.16	3.69	15.38	14.37	16.66	30.50

6	48.80	21.37	23.31	41.83	29.16	31.25
8	0.58	46.17	36.77	36.27	22.02	31
9	1.16	53.03	39.42	32.67	26.78	42.75
10	3.48	62	43.50	26.79	32.73	48
11	18.60	64.11	47.59	17.64	35.71	61.75
13	54.84	33.50	0.72	16.01	18.75	13.75
14	–	44.06	15.62	–	1.48	–
15	–	26.28	1.92	–	2.97	–
16	26.42	31.92	0.48	34.64	0.89	29.50

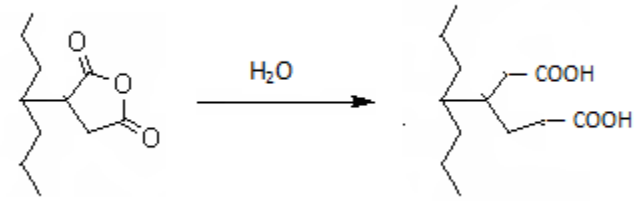


Table (9) indicate the best sample used in extraction of specific amino acid which had been chosen to complete the study. The results illustrate that the type of the chosen sample depends on the type of the amino acid .For example Lysine and Arginine have basic nature , have been extracted more efficiency by sample (11)which was grafted with 41%of succinic anhydride. In other hand Aspartic acid which had acid nature was preferred to be extract by sample 6 which was grafted with 41% NPM .In contrast the presence of aromatic ring within the chemical structure of L-phenyl alanine and L-Tyrosine led to increase the basicity of the amine moiety of the amino acid and increase their ability to link with succinic group grafted to PE of sample (11).From these observation we can conclude that the acid type amino acids are best extracted by polymer grafted with high concentration of maleimide , whereby the basic amino acid are preferred to be extracted by polymer grafted with high concentration of succinic anhydride .

Table (9): Chosen polymer sample with higher efficiency of extraction the amino acids

Amino acid	Best polymer sample	%Extraction
glycine	13	54.84
L-phenyl alanine	11	64.11
L-tyrosine	11	47.59
DL-aspartic	6	41.83
L-lysine	11	35.71
L-arginine	11	61.75

Analytical study:
Effect of amino acid concentration:

Tables (10) give the percent of amino acid extracted by the chosen polymeric sample from their aqueous solutions with different concentrations. It is clearly noticed that the efficiency of extraction was increased by decreasing the concentration of the original amino acid solution except in case of glycine amino acid.

Table (10): Percent of amino acid extracted by 0.1g polymer from their aqueous solutions with different concentrations at 35^0C

Amino acid	Solution concentration				
	500 ppm	200 ppm	100 ppm	50 ppm	25 ppm
L-phenyl alanine	64.11	65.69	66.49	67.54	75.13
L-tyrosine	47.59	49.51	54.56	62.25	67.78
L-lysine	35.71	39.28	47.02	61.30	64.28
L-arginine	61.75	63.50	69.25	75.75	79
DL-aspartic	41.83	47.71	57.51	61.76	66.66
Glycine	54.84	51.66	37.85	27.85	19.04

Effect of pH:

The chosen polymeric sample have been treated with the solutions of amino acids with 25ppm concentration at different pH for 25hrs at$35C^0$. Table (11) shows the results of extraction. The results indicates that the largest amount of amino acids have been taken by the polymer sample was at a normal pH of the amino acids solutions.

Table (11): Effect the pH of the amino acid solution on extraction efficiency

Amino acid	pH					
	2	4	*aqueous solution	7	10	11
L-phenyl alanine	14.81	34.92	75.13	64.02	43.91	39.68
L-tyrosine	19.71	39.90	67.78	63.94	60.09	48.07
Glycine	4.76	7.85	37.85	36.90	25	12.85
L-lysine	23.80	27.97	64.28	36.30	55.95	44.04
L-arginine	51	55	79	59	64.5	61.5
DL-aspartic	59.47	61.43	66.66	58.16	50.98	50.32

*

Amino acid solution	L-phenyl alanine	L-tyrosine	glycine	L-lysine	L-arginine	DL-aspartic
pH	6.21	6.94	6.17	9.53	9.60	4.26

The data indicate that the efficiency of extraction was influenced by the pH of the medium. The neutral amino acid (L-phenyl alanine , L- tyrosine and glycine) were extracted more efficiency in basic medium .This can be explained by that the linkage of the polymer with amino acid is through the amine site of the amino acid which was affected by the acidity of the medium and convert to ammonium group . On the other hand, in case of the basic amino acid (L- lysine and L–arginine) it was noticed that the basic medium decrease the efficiency of extraction. The explanation may be belong to the competitive reaction between the basicity of the medium and the amino acid to react with carboxyl group grafted on the polymer.

Effect of the treatment time:

The effect of the treatment time on the extractability at optimum working pH for amino acids with chosen polymers are shown in table (12) .The results show that the reaction process reach equilibrium state at finite time . The maximum time needed for reaching the equilibrium state is about (2hrs).

Table (12): Treatment time effect on extraction efficiency polymer

Amino acid	Treatment time							
	$\frac{1}{4}$ hr	$\frac{1}{2}$ hr	1hr	$1\frac{1}{2}$ hr	2hr	4hr	16hr	24hr
L-phenyl alanine	15.87	28.04	59.78	71.42	75.13	75.13	75.13	75.13
L-tyrosine	19.71	39.42	51.92	59.61	67.30	67.78	67.78	67.78
L- lysine	39.28	48.21	55.95	61.90	64.28	64.28	64.28	64.28
L-arginine	49	63	74.5	76	79	79	79	79
DL-aspartic	35.94	38.56	47.71	57.51	66.01	66.66	66.66	66.66
Glycine	2.61	8.80	22.85	25.95	35.95	37.85	37.85	37.85

Effect of temperature:

The effect of temperature on the extraction efficiency was studied at optimum conditions. The results were shown in table (13). It was noticed that the efficiency of extraction was increased with temperature elevation with different ratios according to the type of amino acids.

Table (13): Effect of temperature on extraction efficiency

Amino acid	temperature				
	10°C	25°C	35°C	50°C	60°C
L-phenyl alanine	35.44	51.85	75.13	76.71	77.77
L- tyrosine	59.61	61.53	67.30	72.11	75.96
L- lysine	39.28	58.92	64.28	67.26	71.42
L-arginine	66.5	71.5	79	80	82
DL-aspartic	49.01	61.43	66.01	66.66	67.32
Glycine	8.57	16.90	37.85	40	42.38

Loading capacity of the chosen polymers:

The loading capacity of the polymer is the number of mellieqvalent of amino acids extracted by 1 gram of the polymer. Table (14) indicate the loading capacity of studied polymers at different concentration of amino acid solutions. It is clearly showed that the capacity was increased with the dilution of the solutions of all amino acids accept that of glycine amino acid. The result in the table indicates that sample 11, which was grafted with maleic anhydride have higher capacity. This means that succinic group is more efficient in extraction than the maleimide group. It can be seen that the loading capacity with different amino acids have the following order:
L –arginine > L-phenyl alanine > L-tyrosine> L-lysine

Table (14): Loading capacity of the chosen polymer (meq/g) at different concentration of amino acid's solutions

Amino acid	Polymer capacity (meq/g)			
	200 ppm	100 ppm	50 ppm	25 ppm
L-phenyl alanine	1.50	1.52	1.54	1.71

L- tyrosine	1.13	1.25	1.42	1.55
L- lysine	0.90	1.08	1.40	1.47
L-arginine	1.45	1.59	1.73	1.81
DL-aspartic	1.09	1.32	1.41	1.53
Glycine	2.89	2.11	1.55	1.06

Bonded amino acid recovery:

The absorbents amino acid by the polymer samples have been recovered by treating the loaded polymers with different eluents according to the type of amino acid .Hydrochloric acid was used to recover L- phenyl alanine, L- tyrosine and L-lysine, while phosphoric acid was used to recover L- arginine .To recover DL- aspartic acid and glycine we used ammonium hydroxide as an eluent. Many factors have been studied to elucidate the efficiency of recovery process as the concentration of eluents, time of treatment and the temperature.

Effect of eluent concentration:

Different concentrations of eluent were used as shown in tables (15, 16, and 17). The results demonstrate that the efficiency of recovery was increased by increasing the eluent's concentration. The recovery of the amino acids take the following order

L- Phenyl alanine < L-lysine < L-tyrosine < L-arginine < DL-aspartic < glycine

Table (15): Percent of the recovered amino acid by using hydrochloric acid as eluent for 24hrs period of treatment

Amino acid	Eluent' s concentration				
	0.5N	1N	2N	4N	5N
L-phenyl alanine	10.88	12.48	18.45	24.15	31.54
L-tyrosine	20.25	22.2	35.48	42.85	50.85
L-lysine	18.61	26.66	29.16	50.50	_

Table (16): Percent of L-arginine recovered by treatment with phosphoric acid as eluent for 24hrs period of treatment

Amino acid	Eluent' s concentration				
	0.5N	1N	2N	3N	5N
L- arginine	16.66	23.72	27.15	58.16	_

Table (17) :): Percent of the recovered amino acid by using ammonium hydroxide as eluent for 24hrs period of treatment

Amino acid	Eluent' s concentration			
	0.5N	1N	2N	3N
DL- aspartic	_	34.87	55.05	69.23
Glycine	26.25	31.66	60.60	74.25

Time of treatment effect:

Table (18) shows the effect of the time of treatments with the different eluents on recovery efficiency. The results indicate that the time needed to reach the steady state of recovery was about 4hrs for phenyl alanine, tyrosine, lysine and arginine, while it was 2hrs for aspartic and glycine.

Table (18): Percent of amino acid recovered by using the appropriate eluent with better concentration at 35^{O}C for different period of treatment

Amino acid	Period of treatment						eluent
	1hr	2hr	4hr	5hr	16hr	24hr	
L-phenyl alanine	_	19.6	31.32	31.36	31.50	31.54	HCL
L-tyrosine	_	12.78	50.16	50.30	50.40	50.85	HCL
L-lysine	15.31	39.04	50.12	50.16	50.27	50.50	HCL
L- arginine	20.13	24.41	58.04	58.04	58.09	58.16	H_3PO_4
DL- aspartic	43.20	69.09	69.14	69.15	69.20	69.23	NH_4OH
Glycine	38	74	74.03	74.04	74.08	74.25	NH_4OH

Temperature effect

Different temperatures were used for recovery treatment. Table (19) shows the effect of temperature on recovery efficiency. It was noticed that the efficiency of recovery was activated by temperature elevation.

Table (19): Percent of amino acid recovered at different temperature by using the appropriate eluent with better concentration and period

Amino acid	temperature				
	30 C^{o}	40 C^{o}	45 C^{o}	50 C^{o}	60 C^{o}
L-phenyl alanine	31.50	38.29	44.02	51.92	54
L-tyrosine	50	56.45	60.48	62.42	67.19
L-lysine	50	57.40	64	68	75
L-arginine	57.61	58.12	58.35	58.65	60.12
DL-aspartic	69.68	80	86.30	88.21	90.90
Glycine	75.83	76.98	79.45	80.86	94.33

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