

CARBON MONOXIDE CHEMISORPTION-CHARACTERIZATION AND TESTING OF PREPARED NICKEL CATALYSTS FOR AMINATION OF ETHANOL

Achmad Hanafi Setiawan

Research and Development Centre for Applied Chemistry-LIPI
Kompleks PUSPITEK, Serpong, Tangerang 15310.

ABSTRACT

Three types of nickel metal catalyst supported on silica gel have been prepared using impregnation, ion exchanged sodium hydroxide and ion exchanged ammonia methods, in order to investigate the influence of preparation methods on metal dispersion and their activities for amination of ethanol. All the catalyst samples had a nominal nickel loading of 5 % (w/w). After preparation, the catalysts were activated by a drying stage followed by calcination and reduction. The result of transferring the nickel salt to the support phase has shown that the impregnation method was the most efficient, and the least efficient being the ion exchanged sodium hydroxide method. The results from the carbon monoxide chemisorption studies showed that the nickel is poorly dispersed(2%) in the impregnated sample, and highly dispersed(20%) in the ion exchanged sample prepared by the ammonia method. The dispersion was lower(6%) for the catalyst prepared by the sodium hydroxide method due to the formation of sodium nickel silicates which were difficult to reduce. The amination of ethanol over these catalysts was found to take place at 503 °K with methane being formed as a by-product. The ion exchanged sample prepared by the ammonia method gave the highest yield(57%) of ethylamine although its specific activity/m² nickel metal(4×10^{-7}) was similar to the sample prepared by the impregnation method. The retained sodium in the ion exchanged sodium hydroxide catalyst poisoned metal sites for ethanol amination.

INTISARI

Tiga jenis katalis logam nikel dalam silika gel telah dibuat menggunakan metoda impregnasi, pertukaran ion secara natrium hidroksida dan secara amonia untuk meneliti pengaruh perbedaan cara pembuatan pada dispersi logam dan aktifitasnya didalam reaksi aminasi dari etanol. Semua contoh katalis mempunyai kadar nominal nikel 5 % berat. Setelah pembuatan, katalis tersebut kemudian diaktifkan dengan pemanasan pada suhu rendah dan diikuti dengan proses kalsinasi dan proses reduksi. Hasil proses pemindahan garam nikel kedalam suport menunjukan bahwa cara impregnasi merupakan cara yang paling efisien sedangkan cara pertukaran ion – natrium hidroksida kurang efisien. Dari hasil percobaan penyerapan gas carbon monoksida terlihat bahwa dispersi logam nikel pada

contoh katalis yang dibuat dengan cara impregnasi sangatlah rendah(2%), tetapi yang dihasilkan oleh katalis yang dibuat dengan pertukaran ion secara amonia cukup tinggi(20%). Katalis yang dibuat dengan penukar ion cara natrium hidroksida mempunyai dispersi yang masih rendah(6%). Hal ini diperkirakan karena adanya pembentukan natrium nikel silikat. Aminasi alkohol menggunakan katalis logam nikel telah teramati berlangsung pada suhu 503 °K dengan gas metan sebagai hasil sampingnya. Katalis yang dibuat dengan cara penukar ion secara amonia memberikan hasil amina yang paling tinggi(57%) meskipun aktifitas tiap m² logam nikel(4×10^{-7}) sama dengan contoh yang dibuat dengan cara impregnasi. Logam natrium yang masih tersisa pada katalis yang dibuat melalui penukaran ion secara natrium hidroksida diperkirakan meracuni keaktifan katalis pada reaksi aminasi dari etanol.

INTRODUCTION

It has been well documented that the ability of a substance to act as a catalyst in a specific system depends on its chemical nature i.e. the specific chemical properties of the surface of the catalyst(1) where its structure is largely determined by the technique of preparation(2). The preparation techniques involve contact of the support with a solution containing a soluble salt of the metal being added. The impregnation technique involves filling the pores of the support with the metal salt solution (3,4) and the ion exchange technique involves exchange of the ions in the salt solution with acidic protons on the support surface(3,5).

The surface structure and chemical state at the surface is determined from the dispersion of the active component and its coordination geometry (6). Chemisorption of carbon monoxide has been commonly used to probe the surface transition metal ion. It has been found that carbonmonoxide adsorbed onto the surface of nickel metal in a linear and bridge forms. The former is generally more intense in the spectrum than the latter (6,7).

For catalysis to proceed, there must be some surface interaction between the catalyst and the reactant but this interaction must not permanently change the chemical

nature of the catalyst(8). Therefore, in catalytic testing for amination of ethanol, the catalytic activity of the prepared catalysts is determined for a model reaction to obtain information on catalyst performance for the amine products. Aliphatic amines may be prepared by aminating alcohols, aldehydes and ketones in the presence of ammonia and hydrogen over a suitable catalyst at elevated temperatures and pressures (9). Silver, copper and nickel supported catalysts have resulted in a good selectivity for amines (10) forming primary, secondary and tertiary amines preferentially and hydrocarbons, nitriles and ethers as by products. Optimum conditions for the dehydroamination reaction depend on the type of reactor used and the most important parameters which influence the optimum condition are type of catalyst used, reaction temperature, molar ratio and total pressure (2). Supported copper catalysts containing 10-65 % weight copper showed a high yield for the dehydroamination of aliphatic alcohols (11). Silica supported materials have been used in this reaction. The support itself showed no dehydroamination activity. The reaction mechanisms proposed by many workers (9, 12, 13) are specific to a particular catalyst. Sharratt (14) has proposed the amination mechanisms of ethanol using a 10 % nickel supported on silica catalyst prepared by impregnation. He suggested that $\text{CH}_3\text{-CH=}$ was the intermediate species in the reaction which was formed by the dehydration of ethanol.

The aim of this work is to obtain information of the relationship among the three preparation routes (impregnation, ion exchanged sodium hydroxide and ion exchanged ammonia methods) of supported nickel catalyst, their dispersion, nickel metal surface area and catalysis performance for the amination of ethanol.

EXPERIMENTAL

Materials

Nickel(II)nitrate hexahydrate, (Jansen Chimica), and silica gel, Gasil 35, (Crosfield Chemicals), were used as the starting materials. The impurity levels of Gasil 35 are 1000-2000 ppm Al; less than 100 ppm of Mg, Ca and K; 10-100ppm of Fe, Cu, Zn, Sr, Ba, P, S, Cl and F. The silica has a surface area of $300 \text{ m}^2 \text{ g}^{-1}$, with mean pore diameter of 16 nm and mean pore volume of $1.55 \text{ cm}^3 \text{ g}^{-1}$ (15).

Helium(BOC Ltd) was used as the carrier gas for gas chromatography; Hydrogen(BOC Ltd) was used to reduce the catalyst and as the gas supply for the flame ionisation detector(FID); Air(BOC Ltd) was also used for FID analysis; 5 % ammonia in hydrogen (G&E, Union carbide Ltd) and ethanol (BDH) were used as reactants.

Catalyst preparation

Nickel supported on silica catalyst precursors were prepared using either impregnation or ion exchange

method. Ion exchanged samples were prepared using either sodium hydroxide or ammonium hydroxide as the base. For the impregnation, silica gel (10 g) was mixed with distilled water to make a homogeneous slurry and 30 ml of 0.28 M nickel salt solution in water was added to the slurry. The mixture was stirred for 30 minutes and was then left standing for about 120 minutes to allow both materials to reach equilibrium. Excess water was then removed on a rotary evaporator and the catalyst precursor was dried in an oven at 383°K . Finally, the precursor was calcined at 673°K for four hours in a furnace. In the ion exchange sodium hydroxide method, the ion exchange column was prepared first by pouring a slurry (a mixture of one part silica gel and two parts water) along the wall of the column allowing the particles to settle forming the ion exchange bed. During this stage water was constantly passed through the column with the excess solution being drained off at 1-2 ml per minute. The column was then treated with dilute sodium hydroxide solution (pH about 9) about 30 minutes and washed with distilled water until the filtrate neared neutrality. Afterwards, the ion exchange column was eluted with 30 ml of a 0.28 M nickel salt solution in water as used in the impregnation method and the excess solution was drained off at a flow rate of 1-2 ml per minute. The column was then washed with distilled water to remove all the free metal salt. The solid obtained was dried at 383°K overnight. Finally, the dried sample was calcined at 673°K in a furnace for four hours. In the ion exchange ammonia method, 0.28 M nickel hexamine solution was prepared by dissolving nickel(II)nitrate hexahydrate in 30 ml of 3 M ammonia solution. This nickel salt solution was then passed through the ion exchange bed which was prepared as in the sodium hydroxide technique and the excess solution was again drained off at 1-2 ml per minute. Next, the column was washed with distilled water to remove all free metal salt. The solid precursor was dried overnight at 383°K and was then calcined at 673°K in a furnace for four hours.

Chemical analysis of the precursors

The bulk composition of the catalyst was investigated using Perkin-Elmer M 1100, atomic absorption spectrophotometer. The sample preparation and calculation described by Mile *et.al.*(15) were used.

The percentage of sodium retained in the ion exchanged sample using the sodium hydroxide method was determined by flame photometry using a Corning 410 flame photometer.

Carbon monoxide chemisorption

The apparatus used is shown schematically in Figure 1. The operation procedure and the results calculation described by Brooks and Christopher(16) were used.

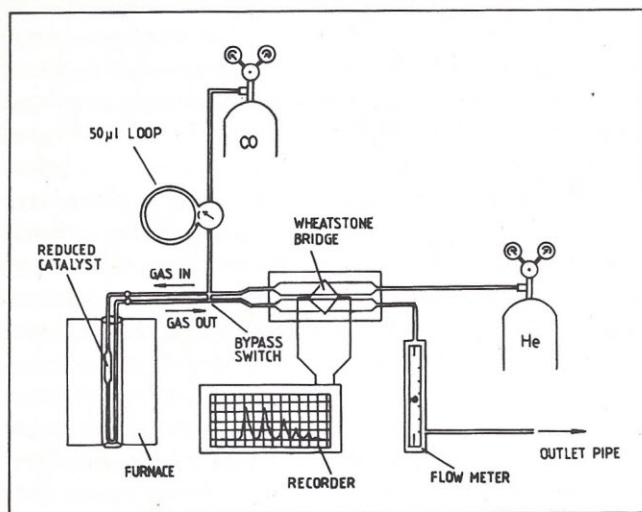


Figure 1 : Schematic diagram of chemisorption apparatus.

The amination of ethanol

The initial part of this study was concerned with the design, construction and calibration of a microreactor operating at a temperature of 383 - 573 °K, pressure of approximately 20 psi and a contact time of 20 minutes. A microcatalytic reactor system coupled to a gas chromatograph which its design was explained by Sharratt(14), was used throughout this study as illustrated in Figure 2.

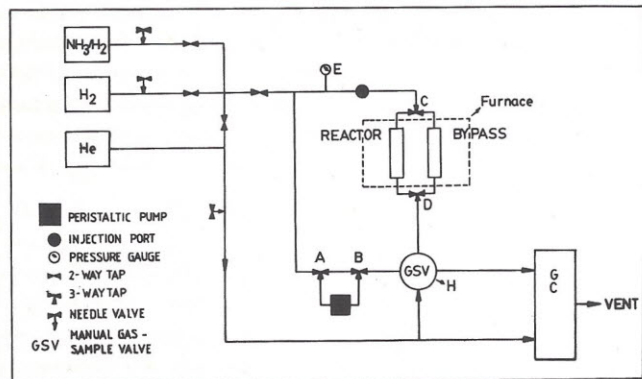


Figure 2 : Schematic diagram of the apparatus for amination reaction.

Preparation of samples: 0.2 g of the press-calcined impregnated and ion exchanged catalysts were loaded into the 6 mm reactor used for catalyst testing. The catalyst was initially purged with He gas at 25 ml/min for one hour prior to reduction in hydrogen at 25 ml/min, programmed from room temperature to its reduction temperature. The temperature was maintained for three hours and then the catalyst was cooled in flowing hydrogen. The reactor was filled with 20 psi of hydrogen before it was by-passed.

Test reaction: The line was heated to 383° K and swept with pure hydrogen several times. It was then filled with 5 % of ammonia in hydrogen to 20 psi. 100 µL pure ethanol was then injected into the septum and the line

turned on to the circulating mode. The gaseous mixture was circulated at 15 ml/min for 15 minutes, whilst the gas chromatograph was prepared for use and the catalyst-bed heated to the reaction temperature of 503 °K in helium. The reaction mixture was then passed over the catalyst for 20 minutes. 100µL samples were withdrawn and analysed by gas chromatograph.

RESULTS AND DISCUSSION

The results of the nickel and sodium content of the catalyst precursors are given in Table 1.

Table 1 : The nickel and sodium content (% w/w) in solid catalyst precursors measured by atomic absorption spectroscopy and flame photometry respectively.

Preparation Method	% Nickel	% Sodium
Impregnation	5.29	—
Ion exchanged sodium hydroxide	3.37	0.09
Ion exchanged ammonia	4.78	—

It can be seen from the table that the impregnation technique was more efficient than the ion exchange in transferring the nickel salt to the support phase. This might be due to the step of washing process which present only in the ion exchange technique where the free nickel salt was removed from the precursor. The sodium hydroxide ion exchanged technique was the least efficient where 0.09 % of the sodium was left in the precursor.

The results of estimating of the total nickel metal surface area and dispersion for each catalyst are shown in Tables 2 and 3.

Table 2 : Total nickel surface area of impregnated and ion exchanged samples based on the chemisorption of carbon monoxide.

Samples	Total nickel metal surface area m ² / g
Impregnated	14.32
Ion exchanged sodium hydroxide method	38.01
Ion exchanged ammonia method	131.24

Table 3 : The percentage nickel dispersion of impregnated and ion exchanged samples based on the chemisorption of carbon monoxide.

Samples	% Dispersion	
	Xm=1	Xm=2
Impregnated	2.15	1.07
Ion exchanged sodium hydroxide method	5.71	2.85
Ion exchanged ammonia method	19.70	9.85

Note: Xm is the average number of surface nickel metal sites associated with the adsorption of one or two carbon monoxide molecules.

The ion exchanged samples have both higher total nickel metal surface areas and dispersion percentage than the impregnated sample. With ion exchange technique, the ions of the metal precursor were strongly bound to the carrier and could be expected to be atomically dispersed on the surface. Little migration would then be expected to occur during drying and calcination. In the case of impregnation technique, the nickel(II) ion adsorbed is weakly held by the carrier, so that migration and aggregate formation may readily take place during drying and calcination. The poor dispersion of the ion exchanged sample prepared by the sodium hydroxide method may be due to sodium poisoning of the metal sites (17).

The results of blank experiment in which ethanol, hydrogen and ammonia were circulated through the peristaltic pump in the absence of the catalyst, are shown in Figure 3.

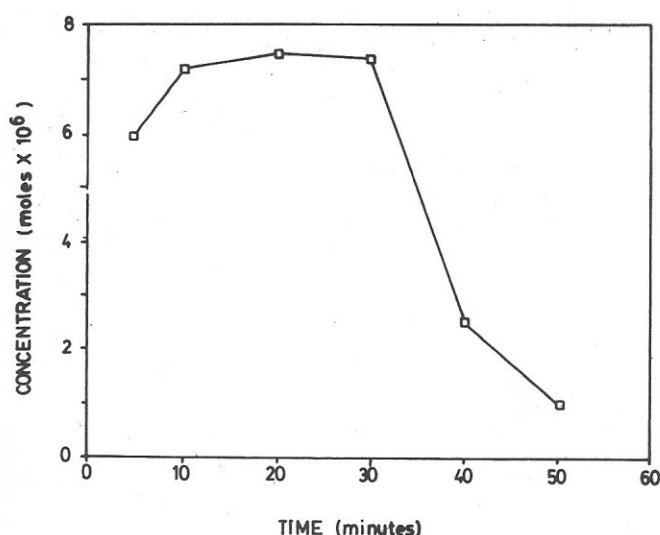


Figure 3 : Ethanol concentration against the circulated times.

The highest ethanol concentration was obtained from the system when the ethanol was circulated for between 10 and 30 minutes. As a result of this study, the reactants were circulated for 10 minutes before being admitted to the catalyst and 20 minutes circulating over the catalyst. This is in agreement with Sharatt's work who found that the silicon tubing absorbed both some ammonia and ethanol at a certain time, although the circulating reactant mixtures for 26 minutes over a 1 % silica supported nickel catalyst prepared by impregnation method gave a 61 % conversion of ethanol to ethylamine (14).

The studies of ethanol amination in the temperature range of 423 to 573 °K over the reduced catalyst prepared by impregnation method (at 16×10^{-4} moles of ethanol as the initial concentration) are shown in Figure 4.

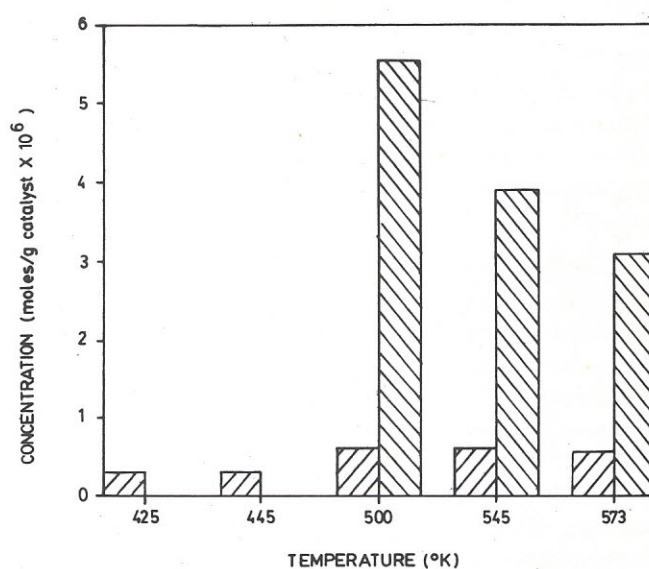


Figure 4: Concentration of methane (▨) and ethylamine (▩) produced against reaction temperature of the amination of ethanol over the catalyst prepared by impregnation method (with reaction mixtures containing 16×10^{-4} moles of ethanol).

At 503 °K the ethylamine was formed, however it decreased as the temperature was further increased. This observation was also reported by Baiker *et.al.* (2) who calculated that the dehydroamination of octanol with dimethylamine in the gas phase was an exothermic reaction. On the basis of these results, the ethanol amination reaction was carried out at 503 °K and the experiment was repeated several times to check for consistency.

The reduced ion exchanged catalysts did not form any ethylamine after the standard reduction in hydrogen gas at 673 °K for three hours. These catalysts were therefore reduced at the higher temperature of 773 °K.

A further blank experiment was carried out in which ethanol in a hydrogen atmosphere was passed over the impregnated catalyst at 503 °K in the absence of ammonia.

The results in Figure 5 shows that the catalyst formed only methane presumably by the cracking of ethanol.

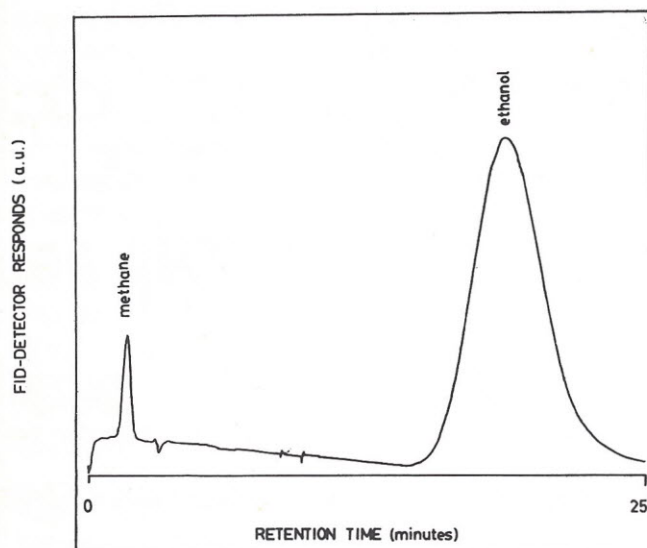


Figure 5: Chromatograms of the ethanol blank experiment (ethanol in hydrogen atmosphere over the catalyst prepared by impregnation method) measured by using Gas Chromatograph PE 8500 Series.

The results for the ethanol amination reaction over the three catalysts prepared by either impregnation or ion exchange are shown in Table 4 while Table 5 and 6 show the number of moles of ethylamine and methane formed, yield percentage of ethylamine and yield percentage of methane respectively, calculated per g catalyst, over catalysts prepared by impregnation and ion exchange methods. Table 7 shows the ratio of moles methane formed in the ethanol/hydrogen blank experiment and ethanol amination reaction over the different catalysts.

Table 4: Moles of ethylamine and methane per g catalyst formed in the ethanol amination reaction over catalyst prepared by impregnation and ion exchange methods.

Preparation method	Moles of ethylamine per g catalyst	Moles of methane per g catalyst
Impregnation	6.10×10^{-6}	0.4×10^{-8}
Ion exchanged sodium hydroxide	5.80×10^{-6}	1.8×10^{-8}
Ion exchanged ammonia	59.25×10^{-6}	4.0×10^{-8}

Table 5: Moles of ethylamine formed and ethanol consumed per g catalyst in the ethanol amination reaction over the catalysts prepared by impregnation and ion exchange methods.

Preparation method	Moles of ethanol consumed per g catalyst	Moles of ethylamine produced per g catalyst	% yield of ethylamine
Impregnation	0.22×10^{-4}	6.10×10^{-6}	27.73
Ion exchanged sodium hydroxide	0.23×10^{-4}	5.80×10^{-6}	25.22
Ion exchanged ammonia	1.04×10^{-4}	59.25×10^{-6}	56.99

Table 6: Moles of ethanol consumed and methane produced per g catalyst in the ethanol amination reaction over catalysts prepared by impregnation and ion exchange methods.

Preparation method	Moles of ethanol consumed per g catalyst	Moles of methane produced per g catalyst	% yield for methane
Impregnation	0.22×10^{-4}	0.4×10^{-8}	0.22
Ion exchanged sodium hydroxide	0.23×10^{-4}	1.8×10^{-8}	0.08
Ion exchanged ammonia	1.04×10^{-4}	4.0×10^{-8}	0.04

Table 7: The mole ratio of methane per g catalyst formed in the ethanol/hydrogen blank experiment and ethanol amination reaction over catalyst prepared by impregnation and ion exchange methods.

Preparation method	Moles of methane per g catalyst formed in the ethanol blank experiment	Moles of methane per g catalyst formed in the ethanol amination reaction	Ratio
Impregnation	0.2×10^{-8}	0.4×10^{-8}	2.0
Ion exchanged sodium hydroxide	0.5×10^{-8}	1.8×10^{-8}	3.6
Ion exchanged ammonia	0.6×10^{-8}	4.0×10^{-8}	6.7

Table 4,6 and 7 indicate that the amination reaction produced more methane than the blank experiment. Therefore, methane was the sole by-product of the amination reaction. The results in Table 4 and 5 demonstrate that the ion exchanged sample prepared by the ammonia method gave the highest yield of ethylamine. The ion exchanged sample prepared by the sodium hydroxide method only produced ethylamine, after the same catalyst sample had been tested twice with fresh ethanol in circulating ammonia/hydrogen mixture. The yield of ethylamine was similar to that obtained using the catalyst prepared by the impregnation technique. Carbon monoxide chemisorption studies showed that the nickel metal dispersion was only 5.7 % and this can be correlated with retained sodium and its effect on the reduction of the catalyst. The percentage yield of ethylamine obtained with this catalyst after treatment with two aliquots of reactant may be due to : i) restructuring of the surface after the first exposure to the reactants resulting in the exposure of fresh active sites for the reaction; ii) increased effective residence time of the reactants on the active sites; iii) the reaction taking place on an adsorbed carbonaceous overlayer (18,19). This catalyst had a nickel content of only 3.4 % compared with 5.3 % for the impregnated and 4.8 % for the ion exchanged ammonia sample. It also contained 0.09 % by weight of sodium which could behave as a poison in this reaction.

Table 8 : Moles of ethylamine and methane per m² nickel based on the nickel metal areas estimated by carbon monoxide chemisorption for the catalyst prepared by impregnation and ion exchange methods

Preparation method	Moles of ethylamine per m ² nickel metal	Moles of methane per m ² nickel metal
Impregnation	4.26 x 10 ⁻⁷	2.79 x 10 ⁻¹⁰
Ion exchanged sodium hydroxide	1.53 x 10 ⁻⁷	14.74 x 10 ⁻¹⁰
Ion exchanged ammonia	4.44 x 10 ⁻⁷	3.01 x 10 ⁻¹⁰

When the product yields are calculated on a metal surface area basis (Table 8), it can be seen that the ion exchanged sample prepared by the sodium hydroxide method was less selective for ethylamine formation and produced more methane per m² compared to the ion exchanged ammonia and impregnated samples. The table also shows that the impregnated and ion exchanged ammonia method catalysts produce virtually equal amounts of ethylamine per m² suggests that the selectivity to ethylamine in the amination reaction is related to the number of nickel sites available.

Table 9: The yield of ethylamine for each of the prepared catalysts compare with their percent dispersion

Preparation method	% Dispersion	% Yield to ethylamine
Impregnation	2.15	27.7
Ion exchanged sodium hydroxide	5.71	25.2
Ion exchanged ammonia	19.70	57.0

Table 9 shows the dispersion percentage based on the carbon monoxide chemisorption, and the yield percentage calculated assuming that the ammonia and hydrogen concentrations remained effectively constant during the reaction. Comparison of the conversion percentage to ethylamine for the catalysts prepared by impregnation and ion exchange methods, clearly shows that the ion exchanged catalyst prepared by the ammonia method is the most selective for ethylamine formation.

CONCLUSIONS

The following conclusions could be drawn from the present investigation:

1. The dispersion of Ni(II), total nickel metal surface area and catalysis performance for the amination of ethanol are all affected by the preparation route.
2. The yield of ethylamine in the amination reaction is related to the number of nickel sites available in each catalyst.
3. The ion exchanged catalyst prepared by the ammonia method is the most selective for the amination of ethanol.
4. The retained sodium in the ion exchanged sodium hydroxide catalyst poisoned nickel metal sites.

ACKNOWLEDGEMENT

The author expresses his appreciation to Prof.G.Webb and Dr.D.Stirling of Department of Chemistry, Glasgow University, U.K. for helpful discussion and to the British Council and P3KT-LIPI for opportunity to pursue the higher degree at the university.

REFERENCES

1. M.A.Kohler, H.E.Curry-Hyde, A.E.Hughes, B.A.Sexton and N.W.Cant., Structure of copper/silica catalysts prepared by the ion-exchange technique, *J.Catal.*, 108, 323-33(1987).
2. A.Baiker and J.Kijensi, Catalytic synthesis of higher aliphatic amines from the corresponding alcohols, *Catal.Rev.-Sci.Eng.*, 27, 653 (1985).
3. R.L.Burwell, G.Pearson, G.I.Tjok and S.P.Chock, The adsorption an reaction of coordination complexes on silica gel, *Inorganic Chem.*, 4, 1123-28 (1965).
4. P.H.Emmet, *Catalysis, Fundamental Principles*, vol.I; Rein hold: New York, (1954).
5. F.A.Cotton and G.Wilkinson, *Advanced Inorganic Chemistry, A Comprehensive Text*; Wiley: New York, Chichester, 5th ed., 1980, p 744.
6. J.R.Anderson and K.C.Pratt, *Introduction to Characterization and Testing of Catalysts*; Academic Press: Sydney, London, (1985).
7. J.B.Peri, Infrared spectroscopy in catalytic research, *Catalysis Sciences & Technology*, 5, 203-4(1984).
8. S.J.Thompson and G.Webb, *Heterogeneous Catalysis*; Oliver & Boyd, Edinburgh (1968).
9. M.V.Klyuev and M.L.Khidekel, Catalytic amination of alcohols, aldehydes and ketones, *Russ.Chem.Revs.*, 49, 14 (1980).
10. Kirk-Othmer Encyclopedia of Chemical Technology, 2, 3rd ed., A Willey Interscience Pub., New York, 1978, p 16.
11. A.Baiker, W.Caprez and W.L. Holstein, Catalytic amination of aliphatic alcohols in the gas and liquid phases; Kinetics and reaction pathway, *Ind.Eng.Chem., Prod. Res.Dev.*, 22, 217-25, (1983).
12. V.A.Nekrasova and N.I.Shuikin, Catalytic metods of preparing aliphatic and alicyclic amines, *Russ. Chem. Rev.*, 34, 843(1963).
13. E.D.Schwegler and H.Adkins, Preparation of certain amines, *J. Am. Chem. Soc.*, 61, 3499-501 (1939).
14. A.P.Sharratt, Ph.D. Thesis, University of Surrey, Surrey, (1991).
15. B.Mile, D.Stirling, M.Zammit, A.Lovell and M.Webb, The location of nickel oxide and nickel in silica supported catalyst, *J.Catal.*, 114, 217-25 (1988).
16. G.C.Brooks and G.L.M.Christopher, Measurement of the state of metal dispersion on supported nickel catalysts by gas chemisorption, *J.Catal.*, 10, 211-23 (1968).
17. M.Houalla, F.Delannay, I.Matsuura and B.Delmon, Physicochemical characterisation of impregnated and ion-exchanged silica-supported nickel oxide, *J.C.S. Faraday I*, 76, 2128-41 (1980).
18. I.M.Campbell, *Catalysis at Surface*; Chapman and Hall; Cambridge, 229 (1988).
19. G.Webb., The formation and role of carboneous residues in metal catalysed reactions of hydrocarbons, *Catal. Today*, 7(2), 139-55 (1990).

Sambungan dari hal. 5

ACKNOWLEDGEMENT

This investigation was supported, in part, by grant CA-33047 from the National Cancer Institute, Public Health Service, Bethesda, Maryland. LBSK wishes to acknowledge scholarship from Overseas Training Office, General Participant Training Project II (1986-1988), and from Overseas Fellowship Program, World Bank, through the Indonesian State Ministry of Research and Technology (1988-1990), and Teaching & Research Assistantship from University of Illinois, Chicago (1990-1992).

REFERENCES

1. L.M.Perry J.Metzger, *Medicinal Plants of East and Southeast Asia: Attributed Properties and Uses*, MIT Press, Cambridge, Massachusetts, 2, (1980).
2. L.van Puyvelde, N.De Kimpe, J.M.Mudaheranwa, A.Gasiga, N.Schamp, J-P.Declercq and M.van Meerssche, Isolation and structural elucidation of potentially insecticidal and acaricidal Isoflavone-type compounds from *Neorautanenia mitis*, *J. Nat. Prod.*, 50: 349-356 (1987).
3. M.E.Oberholzer, G.J.H.Rall and D.G.Roux, The occurrence of 12a-hydroxy- and 12a-O-methylrotenoids, *Tetrahedron Lett.*, 25: 2211-2214 (1974).
4. F.Abe, D.M.X.Donnelly, C.Moretti and J.Polonsky, Isoflavonoid Constituents from *Dalbergia monetaria*, *Phytochemistry*, 24: 1071-1076 (1985).
5. D.W.Shen, A.Fojo, J.E.Chin, I.B.Roninson, N.Richert, I.Pastan and M.M.Gottesman, Human multidrug-resistant cell lines: Increased mdr 1 expression can preceed gene amplification, *Science* 232: 643-645 (1986).
6. R.I.Geran, N.H.Greenberg, M.M.McDonald, A.M.Schumacher and B.J.Abbott, Protocols for screening chemical agents and natural products against animal tumours and other biological systems, *Cancer Chemother. Rep.*, 3: 1-93 (1972).
7. H.Jayasuriya, J.D.McChesney, S.M.Swanson and J.M.Pezzuto, Antimicrobial and cytotoxic activity of rottlerin-type compounds from *Hypericum drummondii*, *J. Nat. Prod.*, 52: 285-331 (1989).
8. J.B.Harborne, *The Flavonoids, Advances in Research since 1980*, Chapman and Hall, pp 152-175 (1988).
9. I.Messoma, F.Ferrari and A.E.Goulart Sant' Ana, Two 12a-hydroxyrotenoids from *Boerhawia coccinea*, *Phytochemistry*, 25: 2688-2689 (1986).
10. D.G.Carlson, D.Weisleder and Tallent, W.H., NMR Investigation of rotenoids, *Tetrahedron*, 29: 2731-2741 (1973).
11. E.Dagne, A.Yenesew and P.G.Waterman, Flavonoids and isoflavonoids from *Tephrosia fulvinervis* and *Tephrosia pentaphylla*, *Phytochemistry*, 28: 3207-3210 (1989).