Concours externe de recrutement de personnel ITRF Session 2011 **Assistant Ingénieur - BAP B - Externe** Emploi type : Assistant en techniques de sciences des matériaux / caractérisation **Epreuve d'amissibilité** ***Durée de l'épreuve 3 h – coefficient 4***	Etablissement organisateur : INSTITUT NATIONAL POLYTECHNIQUE DE LORRAINE 2, avenue de la Forêt de Haye 54501 VANDŒUVRE LES NANCY

Note aux candidats :

- **Votre identité ne doit figurer que dans la partie supérieure de la bande en-tête des copies modèles C que vous utiliserez. Toute autre mention de votre identité ou signature entraînera l'annulation de votre épreuve.**
- **Le sujet est volontairement long mais aucune question n'est éliminatoire.**

  Les réponses seront données directement sur le sujet.
- **Le barème est sur 106 points ramenés à 100 points.**
- **Aucun document n'est autorisé à l'exception d'une calculatrice non programmable.**

Partie I : Hygiène et sécurité (14 points)

1. Qu'est ce qu'un EPI ? Donner trois exemples. (2 points)

2. Avec quel extincteur éteint-on un feu d'origine électrique ? (1 point)

3. Dans quel type de récipient prépare-t-on une solution d'acide fluorhydrique? (1 point)

4. Peut-on stocker ensemble (justifier) : (1 point)
   -de la soude et de la potasse ?

   -de l'acide chlorhydrique et de la potasse ?

5. Peut-on manipuler sans précautions particulières de la poudre de silice ? de la poudre fine d'aluminium ? pourquoi ? (2 points)

6. Que doit-on faire en cas de brûlure ? (1 point)

7. Quels risques peuvent présenter le stockage et la manipulation d'azote liquide ? (1 point)

8. Donnez la signification des symboles suivants (4 points)

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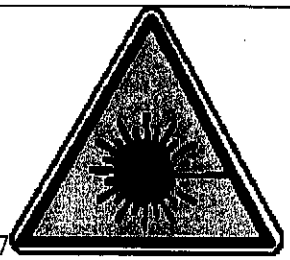
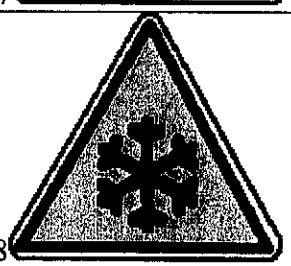
7 	
8 	

9 - Indiquez une méthode pour diluer de la soude concentrée. (1 point)

Partie II : Culture scientifique (16 points)

1. Que signifient les acronymes suivants ? (3 points)

   UMR

   CNRS

   AI

   BAP

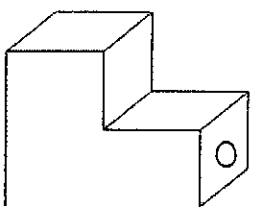
   INPI

   CHS

2. Citez 3 scientifiques français ayant reçu un prix Nobel ces 20 dernières années. (2 points)

3. Définissez la rhéologie (1 point)

4. Dessinez les cinq vues de la pièce suivante : (3 points)



Le trou est traversant

Vue de face : Vue de gauche : Vue de droite :

Vue de dessus : Vue de dessous :

5. Donnez les sept unités primaires du système international (2 points)

6. Exprimez en mètres : (3 points)

   Mégamètre

   Micromètre

   Nanomètre

   Kilomètre

   Gigamètre

   Picomètre

7. Quelle est la gamme de longueurs d'onde des ultra-violets et des infrarouges ? (2 points)

Partie III: Physique (25 points)

1. Quel est le matériau le plus efficace pour atténuer les rayons X ? (1 point)

2. Les rayonnements ionisants sont dangereux. Doit-on aussi prendre des précautions particulières avec un faisceau de rayonnement non-ionisant ? (1 point)

3. Qu'est ce qui différencie deux isotopes ? (1 point)

4. Comment peut-on analyser un mélange de deux isotopes ? (1 point)

5. Définir la radioactivité α, β^-, β^+ et l'émission γ et indiquez les moyens de s'en protéger. (2 points)

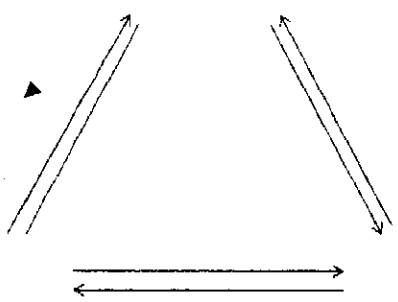
6. Définir isolant, conducteur, semi-conducteur (1 point)

7. Tout atome est caractérisé par son nucléide ${}^{A}_{Z}X$. Définir A, Z et X (2 points)

8. Qu'est ce que la longueur d'onde ? (1 point)

9. Qu'est ce qu'un vide primaire (gamme en Pa)? Quel type de pompe utiliseriez-vous pour l'obtenir ? Mêmes questions pour un vide secondaire. (4 points)

10. Indiquez sur le schéma suivant les trois états de la matière ainsi que les noms des six changements d'état. (3 points)



11. Précisez les différences entre la microscopie optique et électronique (2 points)

12. Quelle technique utiliseriez-vous pour : (4 points)
-observer un monocristal de 200µm de diamètre

-observer des diamants de 5µm de diamètre

-trouver la structure cristallographique d'un échantillon (2 réponses possibles)

-mesurer la hauteur d'une marche

13. Deux voitures sont à une distance de 1 km. La voiture de devant roule à 50 km/h et celle de derrière à 60 km/h. Quelle distance devra parcourir la deuxième voiture et en combien de temps pour rattraper la première ? (2 points)

Partie IV : Chimie (18 points)

1. Citez trois facteurs susceptibles d'accélérer une réaction chimique. (3 points)

2. Qu'est ce qu'une réaction exothermique ? (1 point)

3. A quels éléments correspondent ces symboles ? (2 points)

   F

   Mn

   Zn

   Ar

   S

   Be

   Sn

   Au

4. Qu'est ce qu'un polymère ? (1 point)

5. Qu'est ce qu'une molécule chirale ? Citez un exemple (2 points)

6. Que représente le pH ? (1 point)

7. Classez par ordre de polarité : (2 points)

Dichlorométhane, Eau, Acétonitrile, THF, Acétone, Cyclohexane

8. Citez différents types d'eau purifiée et les moyens pour les obtenir (2 points)

9. Vous devez réaliser un mélange stœchiométrique avec 15 g de $C_{12}H_{18}O_2$ et du $C_7H_{11}O_3N$ de densité 1.06. Quel volume de $C_7H_{11}O_3N$ devez-vous utiliser ? (4 points)

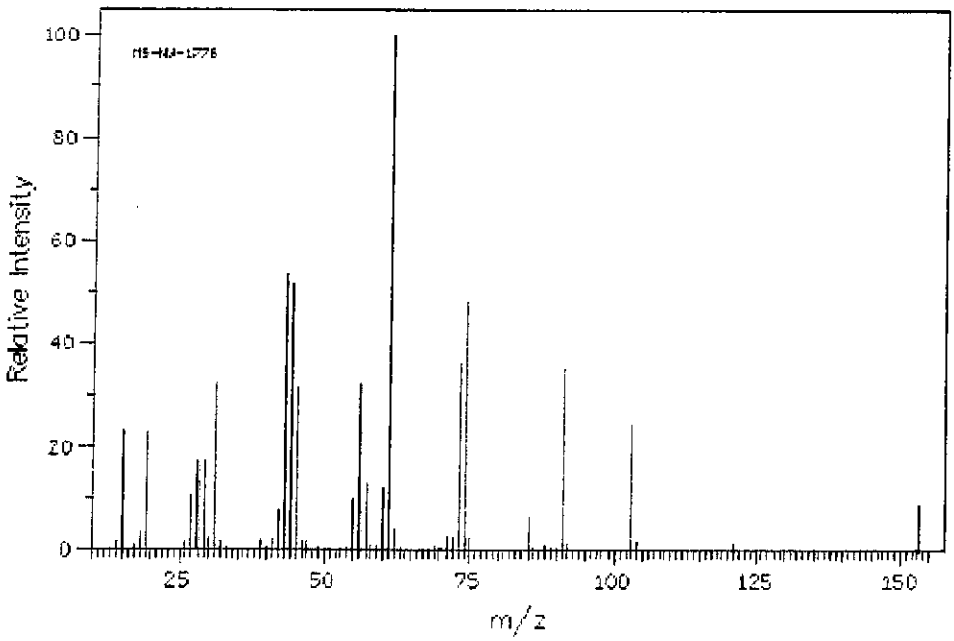
Partie V : Techniques d'analyse chimique (12 points)

1. Quel est le principe de l'ICP et son rôle ? Vous pouvez faire un schéma. (5 points)

2. Quels sont les 2 principaux détecteurs utilisés en chromatographie gaz ? (2 points)

3. A quoi sert le couplage GC/MS ? (2 points)

4. Observez le spectre de masse d'une molécule inconnue ci-dessous. Que signifie le rapport m/z ? Quel est le pic de masse ? Quel est le pic le plus abondant ? (3 points)



Partie VI : Dossier (21 points)

Etudiez le document ci-joint (pages 17 à 21) et répondez aux questions suivantes. Notez que certaines réponses se trouvent dans le document mais pas toutes.

1. Que veut dire HPLC et donnez le principe (schéma possible) (5 points)

2. Quels sont les points communs avec les CCM ? Que veut dire CCM ? (2 points)

3. Quels sont les intérêts des phases normales et inverses ? Comment les réalise t-on ? (4 points)

4. Que permet l'ajout d'un tampon dans la phase mobile ? (2 points)

5. A quoi peut servir d'augmenter la température de la colonne ? (2 points)

6. Exercice pratique (6 points)

On dispose d'une chaîne HPLC sur laquelle on peut monter les colonnes remplies des phases stationnaires suivantes :

- Silice
- Silice greffée C18
- Support greffé Ammonium
- Support greffé Sulfonate

On dispose des phases mobiles constituées des mélanges A et B suivants :

A : hydroxyde de tétrabutyl ammonium 2,8 mM, KH2PO4 25 mM	**B** : CH3OH
A : CH3CN	**B** : H2O
A : Cylclohexane	**B** : Acétate d'Ethyl

Pour chaque échantillon ci-dessous, proposez une phase stationnaire et une phase mobile, justifiez.

Echantillon 1

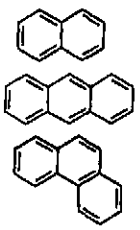
$CH_3CO_2^-$

Cl^-

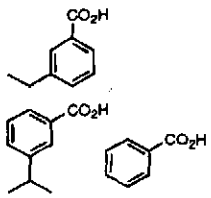
NO_3^-

SO_4^{2-}

Echantillon 2



Echantillon 3



HPLC Columns

The heart of a HPLC system is the column. Changing a column will have the greatest effect on the resolution of analytes during method development. The three main components of an HPLC column are the hardware (column housing), the matrix, and the stationary phase. Column hardware will not be discussed; handling guidelines are included with each column and should be followed for optimal results and column lifetime. Generally, modern reverse phase HPLC columns are made by packing the column housing with spherical silica gel beads which are coated with the hydrophobic stationary phase. The stationary phase is introduced to the matrix by reacting a chlorosilane with the hydroxyl groups present on the silica gel surface. In general, the nature of stationary phase has the greatest effect on capacity factor, selectivity, efficiency and elution[1]

There are several types of matrices for support of the stationary phase, including silica, polymers, alumina, and zirconia. Silica is the most common matrix for HPLC columns.[2] Silica matrices are robust, easily derivatized, manufactured to consistent sphere size, and do not tend to compress under pressure. Silica is chemically stable to most organic solvents and to low pH systems. One shortcoming of a silica solid support is that it will dissolve above pH 7. In recent years, silica supported columns have been developed for use at high pH (*vide infra*).[3]

The nature, shape and particle size of the silica support effects separation. Smaller particle results in a greater number of theoretical plates, or increased separation efficiency. However, the use of smaller particles also results in increased backpressure during chromatography and the column more easily becomes plugged. For this reason 5 Å columns are more frequently used than 3 Å columns in development work. Narrower particle size distribution of the silica particles also results in better resolution. Hence, similar phase columns from different manufacturers, or different lots of columns from the same manufacture may have very different separation properties due to differing methods of matrix preparation.

The nature of the stationary phase will determine whether a column can be used for normal phase or reverse phase chromatography. Normal phase chromatography utilizes a polar stationary phase and a non-polar mobile phase. Generally, more polar compounds elute later than non-polar compounds. Types of columns suitable for normal-phase phase chromatography include underivatized silica, nitrile (*vide infra*), amino (or aminopropyl), glycerol and nitro columns. Chiral separation is usually performed under normal phase conditions.[4] Since highly polar and ionic compounds are retained on normal phase columns, a guard column or silica gel sample purification should be used to extend the column life.[5]

In reverse phase chromatography the stationary phase is non-polar and the mobile phase is polar, causing polar peaks to generally elute earlier than non-polar peaks. To create a stationary phase for reverse phase chromatography on silica support, the free silanols are reacted with a chlorosilane with hydrophobic functionality to introduce the non-polar surface. Due to steric constraints, only about 1/3 of the surface silanols are derivatized. The remaining free silanols can interact with analytes, causing peak tailing. Typically, after the derivitization of a column with the desired stationary phase, the column is further reacted with chlorotrimethylsilane to end cap the remaining free silanols and improve the column efficiency. Common stationary phases are C_4 (butyl), C_8 (MOS), C_{18} (ODS), nitrile (cyanopropyl), and phenyl (phenyl propyl) columns. In general, longer alkyl chains, higher phase loading, and higher carbon loads provide

greater retention of non-polar analytes. Selectivity is most influenced by the amount of accessible surface area of the derivatized silica gel particles and the carbon load. Thus it is often a benefit to not only have columns with different stationary phases, but columns with the same phase from different manufacturers. Commonly used reverse phase columns and their uses are listed below.

Propyl (C_3), Butyl (C_4), and Pentyl (C_5) phases are useful for ion-pairing chromatography (C_4) (*vide infra*) and peptides with hydrophobic residues, and other large molecules. C_{3-5} columns generally retain non-polar solutes more poorly when compared to C_8 or C_{18} phases. Examples include Zorbax SB-C_3, YMC-Pack C4, and Luna C_5.[6] These columns are generally less stable to hydrolysis than columns with longer alkyl chains.

Octyl (C_8 MOS) phases have wide applicability. This phase is less retentive than the C_{18} phases, but is still quite useful for pharmaceuticals, nucleosides, and steroids. Octyl columns are also useful for peptides, peptide mapping and small hydrophilic proteins when bonded to 300 Å silica particles. Examples include (Zorbax SB-C_8, Luna C_8, YMC-Pack-MOS).

Octadecyl (C_{18}, ODS) columns are the most widely used and tend to be the most retentive for non-polar analytes. This phase is useful in ion-pairing chromatography and has wide applicability (same as C_8 in addition to vitamins, fatty acids, environmental compounds). Examples include Zorbax SB-C_{18}, YMC-Pack ODS and Luna C_{18}.

Xterra RP-C_{18} and Zorbax Extend-C_{18} columns have been formulated to tolerate high pH systems (pH >7, normally up to pH 11). Varying the pH can dramatically affect selectivity and resolution of polar analytes, especially for ionizable compounds (*vide infra*).

Phenyl (Ph) columns offer unique selectivity from the alkyl phases and are generally less retentive than C_8 or C_{18} phases. Phenyl columns are commonly used to resolve aromatic compounds. Examples include Zorbax SB-Phenyl, YMC-Pack Phenyl and Luna Phenyl-Hexyl.

Nitrile (CN or cyano) columns are polar and can be used for both reverse and normal phase applications. This phase is often used to increase retention of polar analytes. The nitrile derivatization allows for rapid column equilibration. Examples include Zorbax SB-CN, Luna-CN, and YMC-Pack CN.

Column compatibility with various mobile phases is determined by the stationary phase. In reverse phase chromatography the mobile phase is composed of an aqueous buffer and a water miscible organic solvent that has little or no absorption above 200 nm. The common organic solvents in reverse phase chromatography are methanol, acetonitrile and tetrahydrofuran (*vide infra*). The operational pH range of a column is dependent on the stability of the silica gel support. Special columns with high phase loading, extensive end-capping, or cross-linked stationary phases have been developed for use at pH < 2, or at pH > 7. It is important to check the literature provided with the column for mobile phase compatibility.

Standard C_{18} Columns and similar stationary phases will undergo phase collapse at highly aqueous mobile phases, typically at less than 5-10% organic composition; this will decrease analyte-stationary phase interaction. Collapsed phases are also difficult to re-equilibrate. To prevent phase collapse, C_{18} columns with a polar group embedded in the alkyl chain have been developed to help solvate the hydrophobic chain in >90% aqueous mobile phases.[7] Examples include Zorbax SB-Aq, Synergi Hydro-RP and YMC-Pack ODS-Aq.

Further versatility of C_{18} and C_8 columns can be achieved by utilizing ion-pairing chromatography. In ion-pairing chromatography, ionic modifiers such as tetrabutylammonium or octylsulphonate salts are added to the mobile phase to increase capacity factors for ionic or ionizable compounds[1]. The alkyl chain of the ion-pairing modifier will partition into the hydrophobic stationary phase, producing an ionic surface and leading to increased retention of charged analytes (figure 1).[8] Capacity factors of uncharged analytes will also change slightly due to the change in stationary phase.

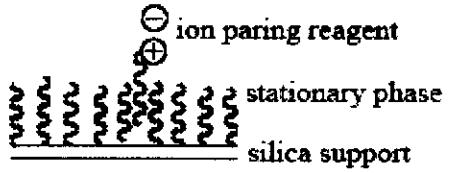


figure 1. ion pairing chromatography

Column temperature control is important for long-term method reproducibility as temperature can affect selectivity.[1] A target temperature in the range of 30–40 °C is normally sufficient for good reproducibility. Use of elevated temperature can be advantageous for several reasons. First, operating at a temperature higher than ambient reduces the viscosity of the mobile phase and thus the overall backpressure on the column. Lower system pressures allow for faster flow rates and thus faster analyses. The temperature may also affect selectivity patterns because analytes will respond dissimilarly to different temperatures. Finally, use of a column oven eliminates variability due to normal fluctuations in the air temperature surrounding the column.

Mobile phase

Though choice of column has the greatest effect on resolution, the mobile phase also effects selectivity and efficiency and is the aspect of chromatography over which we have the most control.[1] In reverse phase chromatography, the mobile phase consists of an aqueous buffer and a non-UV active water miscible organic solvent. The effect of the organic and aqueous phase, and the proportions in which they are mixed will be discussed in this section.

The aqueous buffer serves several purposes. At low pH, the mobile phase protonates free silanols on the column and reduces peak tailing. At sufficiently low pH basic analytes are protonated; when ionized the analyte will elute more quickly but with improved peak shape. Acidic analytes in buffers of sufficiently low pH will remain uncharged, increasing retention. Conversely, at higher pH neutral basic compounds will be more retained, and ionized acidic compounds will elute earlier. Peak splitting may be observed if the pKa of a compound is similar to the pKa of the buffer, and the analyte elutes as both a charged and uncharged species. The pH of a buffer will not greatly affect the retention of non-ionizable sample components.

Typically a 10 – 50 mM solution of an aqueous buffer is used. The most commonly used aqueous phase is a 17 mM solution of H_3PO_4 in water (0.085% v/v H_3PO_4). The pH of a phosphate buffer is easily adjusted by using mono-, di-, or tribasic phosphate salts. However, when phosphate salts are used the solution should be filtered to remove insoluble particles. Other non-UV active acids and bases may also be used to effect differences in peak shape and retention. Table 1 gives a selection of buffers covering a range of pK_a's.

Table 1.

Buffer	pK_a	PH range	UV cutoff
$HClO_4$	– 10		
H_3PO_4/KH_2PO_4	2.12	1.1 – 3.1	<200 nm
KOAc/AcOH	4.8	3.8 – 5.8	210 nm
KH_2PO_4/ K_2HPO_4	7.21	6.2 – 8.2	<200 nm
NH_4OH	9.2	8.2 – 10.2	200 nm
Et_3NH/HCl Et_3NH	11.0	10.0 – 12	<200 nm

Since the degree of solubility of the components of a sample will vary independently in different solvents, the choice of organic solvent can affect selectivity and therefore resolution. In some cases the order of elution of sample components of similar polarity will change with different organic mobile phases. Organic solvents typically used in reverse phase chromatography are methanol, acetonitrile, or THF in order of increasing strength; the strength of a solvent refers to its ability to retain analytes in the mobile phase. Acetonitrile is a highly polar aprotic solvent, providing adequate resolution for many compounds. Due to its ability to form hydrogen bonds, the use of methanol either as an additive or as the organic phase can provide significantly different selectivities. Tetrahydrofuran is the most hydrophobic of the three organic solvents mentioned. Care should be exercised when using tetrahydrofuran in the mobile phase as it is difficult to fully remove from a column. Selectivity is also greatly affected by amount of aqueous solution in the mobile phase, with higher percentages of aqueous phase leading to increased retention and frequently to improved selectivity.

Gradients

Solvent gradients are commonly used in reverse phase chromatography, whereas isocratic methods are commonly used in normal and chiral phase chromatography. Gradient methods permit faster separation with samples containing multiple components of varying degrees of polarity in comparison to isocratic methods, and tend to result in better peak shape for late eluting compounds. Gradient methods typically run from a mobile phase of high aqueous composition to high organic composition, eluting polar compounds before non-polar compounds. When gradient methods are used the column has to be equilibrated to the initial mobile phase composition prior to the next run. When using a standard length C18 column, this can be achieved by programming a 5 – 10 minute post time in the method.

A common gradient method in chemical development runs from 10% to 90% organic over 15 minutes, followed by a 5 minute 90% organic isocratic period. While this gradient is ideal for samples containing a range of polar to non-polar compounds with sufficiently different relative retention times, optimization is needed when this method fails to provide adequate resolution. [1]

In general, a slower ramp rate will give better peak separation but will result in longer run times and may cause peak broadening. Changes made to the beginning of a method will have a greater effect on resolution than those made to the end of method. Therefore, introducing an isocratic period in the early portion of a run, or using slow gradient followed by a steeper gradient is often sufficient to improve resolution while maintaining reasonably short analysis times. Alternately, when a region of poor resolution is identified with respect to solvent composition at time of elution, resolution may be improved by introducing an isocratic region or slower gradient at the appropriate solvent composition. However, in instances where resolution is poor due to poor peak shape resolution may be improved by increasing the ramp rate. Regardless of the method chosen, care should be taken, especially when using isocratic methods, to ensure that all compounds of interest are eluting from the column during the method run time. This may be tested by initially injecting the sample on several different methods or by extending the method run time.

Extraits de « HPLC guide »