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PARTITIONING BEHAVIOUR OF SELECTED ANTIBIOTICS IN ORGANIC SOLVENTS

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ABSTRACT

The commercial production of antibiotics today uses the solvent extraction method in the extraction of antibiotics. Selection of solvent in any solvent extraction process is crucial due to technical and economic reasons. In order to get better understanding on the effect of solvent on the partitioning behaviour antibiotics, selected antibiotics such as kitasamycin, penicillin G, rifampicin and teicoplanin from aqueous buffer solution were studied using various groups of organic solvents such as alcohols, aldehydes, ketones, aliphatic hydrocarbons, kerosene and paraffin, as well as with addition of various extractants in kerosene system in order to enhance solute partitioning. The effects of solvent polarity, pH, antibiotic types and extractant addition on the partitioning behaviour of antibiotics were investigated. The polarity of solvents, in general, increases with an increase in solubility parameter and dielectric constant; and with a decrease in log P. Different groups of organic solvents exhibit different solvent polarity scales. Among them, alcohols are the most favourable solvent group for the partition of antibiotics, followed by aldehydes and ketones. Meanwhile antibiotic types such as kitasamycin and rifampicin displayed a very high degree of partitioning especially for solvent groups of alcohol, aldehyde and ketone over the pH range. On the other hand, addition of the extractant, TOMAC in kerosene displayed excellent partitioning efficiency for all the antibiotics used except kitasamycin whereas AOT was the next superb extractant in kerosene system for all except penicillin G.

Key Words : Extraction, Extractant, Antibiotics, Penicillin, Pharmaceutical.

1.0 INTRODUCTION

Antibiotics, such as penicillins, have a special characteristic that makes them possible to be separated by liquid-liquid extraction. This is because; antibiotics in aqueous solution tend to distribute themselves between the two phases when they are brought into contact with a second immiscible phase. Therefore the desired solute could be separated from a solution with a solvent and thus a purified and concentrated product is obtained [1]. Today the production of antibiotics probably represents the largest single application of liquid-liquid extraction in the pharmaceutical industry [2].

Liquid-liquid extraction or solvent extraction is used by the pharmaceutical industry in a number of ways, some of which are entirely conventional while others have some distinctive features. Conventional liquid-liquid extraction (or physical extraction) is based on the distribution between aqueous and organic phases. This practice has been widely employed for the purification of lipophilic (attracted to or soluble in fats and oils) antibiotics such as penicillins. Solvent extraction method is the preferred choice of extraction in most industrial processes for antibiotics. This method is cost saving due to

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reduction in process time, labour requirements, and energy demand. High selectivity, ease to scale up, equipment simplicity and operation at low temperature (to avoid thermal decomposition) are also factors that make solvent extraction ideal for industrial antibiotic recovery.

However, there are a few drawbacks using organic solvents for extraction. Solvents, for example alcohols, are generally very volatile compounds and can be evaporated easily. This contributes to solvent loss in addition to their mutual solubility in aqueous solution. The desired product may be unstable due to metabolic or microbial action or it may be chemically unstable under the conditions necessary to achieve efficient extraction. Another common problem is the formation of stable emulsion. Besides, conventional solvent extraction has its limitation where it is not applicable for the purification of antibiotics, which are amphoteric and barely soluble in organic solvents.

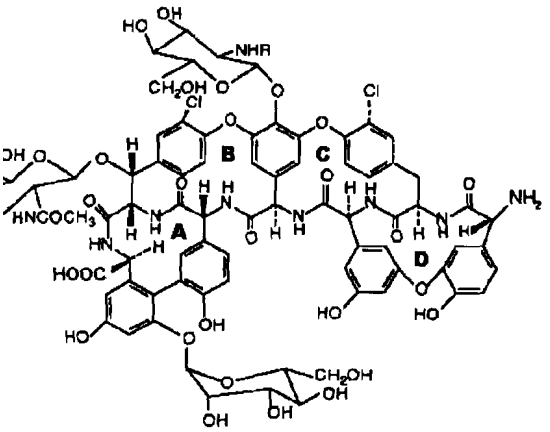
Therefore the successful application of solvent extraction in the separation of antibiotics depends very much on thermodynamic equilibrium constraints such as the partition coefficient of antibiotics between phases, extraction factors and solvent selection, and on process considerations such as the relative flow rates and the extractor used. Moreover, introduction of extractants dissolved in organic solvents can increase the selectivity of extraction and result in much higher partitioning efficiency [3]. According to Johnston [4], the selection criteria for solvent extractants include many factors such as efficiency and selectivity of extraction of the desired species, efficiency and selectivity of stripping, phase separation characteristics, solvent losses and cost.

In this work, we present the results of an experimental investigation of the partitioning behaviour of selected antibiotics with a number of solvent groups (alcohols, aldehydes, ketones, aliphatic hydrocarbons, kerosene and paraffin) and with addition of various extractants in kerosene system. The effects of solvent polarity, pH, antibiotic types and extractant addition on the degree of partitioning were discussed.

2.0 MATERIALS AND METHODS

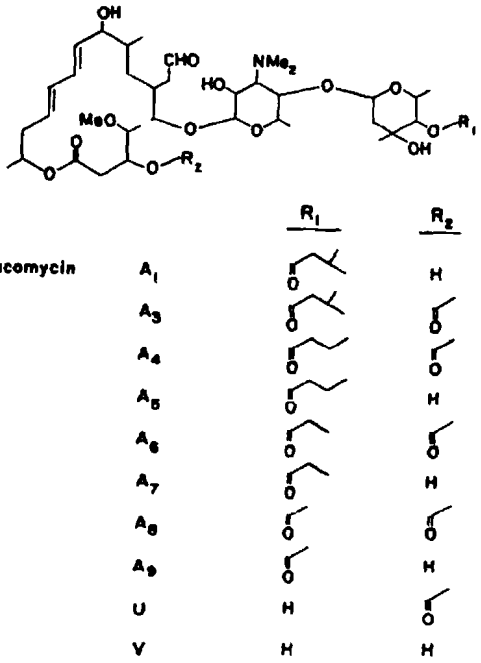
2.1 Materials

Organic solvents of different solvent polarity such as butanol, hexanol, heptanol, octanol, ethyl hexanol, decanol, butyl acetate, pentyl acetate, methyl isobutyl ketone, hexane, heptane, isooctane, cyclohexane, kerosene and paraffin liquid were used in this work. In studying the effect of extractant addition, organic phases were made up of various extractants such as dioctylamine (DOA), trioctylamine (TOA), trioctylmethylammonium chloride (TOMAC) and N-lauryl-N-trialkylmethylamine (AMBERLITE LA-2, hereafter termed as AMBERLITE in this work), as amine extractant group; tributylphosphate (TBP) and diethylphosphoric acid (DEPHA), as phosphorus-bonded oxygen donor (P-O) extractant group; and sodium bis (2-ethylhexyl) sulfosuccinate (AOT), a well-known surfactant to form water in oil microemulsion. All these extractants were dissolved in the solvent, kerosene. Citrate and phosphate buffers were used to make up different pH medium. All chemicals used were obtained from various suppliers with the highest quality available and used as received. Antibiotics employed in this study were teicoplanin, rifampicin, kitasamycin and penicillin G. Figure 1 shows the molecular structures of these antibiotics.

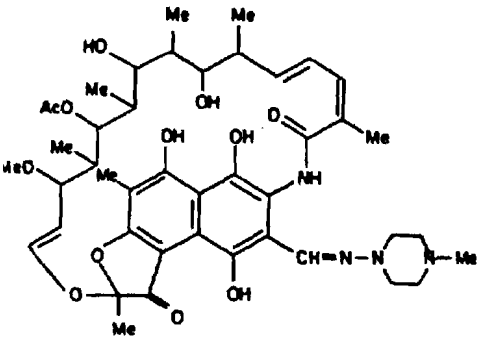


C, D – 4 fused rings
$_3$ – decanoic acid

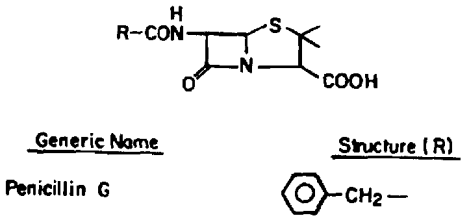
Teicoplanin



Leucomycins (kitasamycins)



Rifampicin



Penicillin G

Figure 1 Structures of selected antibiotics.

2.2.1 Extraction procedures

The extraction experiments were carried out by contacting an equal volume (10 ml) of an aqueous antibiotic solution with an organic solution (various groups of organic solvents or various extractants dissolved in kerosene) in a 30-ml glass bottle. The pH of the aqueous antibiotic solutions was controlled by adding 20% of appropriate 0.1M buffer solutions, which was enough to maintain the pH of the aqueous antibiotic solution after extraction within ± 0.5pH unit. The mixture solution was then mixed using magnetic stirrer at a

speed that provides vortex mixing for 30 minutes to attain equilibrium. All the extractions were carried out at room temperature (25±1°C).

The concentration of antibiotic in aqueous phase was measured using UV/VIS spectrophotometer at the specified wavelength (teicoplanin, 245 nm; rifampicin, 257 nm; kitasamycin, 238 nm; penicillin G, 257 nm). The amount of antibiotic partitioned into the organic solution was determined using mass balance. The pH of the aqueous antibiotic solution was measured using pH meter.

3.0 RESULTS AND DISCUSSION

3.1 Effect of solvent polarity

Solvent polarity is a commonly used term to relate the capacity of a solvent to the solvation of dissolved charged or dipolar species, although the term polarity is difficult to express quantitatively. Often, solvent physical constants have been used to represent solvent polarity. The effects of several physico-chemical constants of solvents, used as a measure of solvent polarity, such as the solubility parameter, *log P* and dielectric constant, on the partitioning behaviour of antibiotics were briefly discussed here.

We first look at the effect of solvent polarity on the partitioning behaviour of antibiotics for alcohols by examining their solvent polarity scales. Table 1 shows that when the molecular weight of the solvents increases from butanol to decanol, there is a decrease in the values of solubility in water and solubility parameter. A similar trend was also observed for dielectric constant whilst a reverse effect was observed for *log P*. The polarity of solvents, in general, increases with increasing solubility parameter and dielectric constant; and with decreasing *log P* [5,6]. Therefore it can be concluded that the solvent polarity increases with a decrease in molecular weight of alcohols, for example, butanol and hexanol with lower molecular weight have higher solvent polarity.

Higher solvent polarity contributes to higher extraction efficiency and superior partitioning behaviour. This is generally true as it complies with the results from Figure 2 which illustrates the effects of *log P*, dielectric constant and solubility parameter on the extent of antibiotic partitioning in organic solvents, taken kitasamycin and penicillin G as the model antibiotics at pHs 5 and 7, respectively. It can be seen that the percentage of partitioning for kitasamycin and penicillin G decreases from butanol to decanol in line with increasing *log P* (corresponding to decreasing solvent polarity). On the other hand, the partitions for both antibiotics increase with an increase of dielectric constant and solubility parameter (an increase of solvent polarity). This is especially apparent for the alcohol group.

Compared to alcohols, solvent groups of aldehyde and ketone show the next highest partitioning capability corresponding to their solvent polarity scales. As shown in Table 1, they occupy the intermediate range of solubility parameter, *log P* and dielectric constant, with light alcohols at optimal conditions and aliphatic hydrocarbons at the pits.

Among the various ketones cited in literature, methyl isobutyl ketone (MIBK) has been used most frequently due to its higher ability of coordination (more basic character of the keto oxygen), higher chemical stability, and a smaller mutual solubility in water [7]. In this study, MIBK displays a very high degree of partitioning especially with kitasamycin and rifampicin which is almost 100% at optimum pH.

Table 1 The solvent polarity scales.

Solvent Groups	Solvents	Solvent Code	Molecular Weight, M_w	Normal Boiling Point, T_n (°C)	Solubility (Water, 20°C) (% W)	Solubility Parameter, δ	*Log P*	Dielectric Constant, ϵ (20°C)
ALCOHOL	Butanol	BUTO	74.12	117.73	7.4	11.4	0.8	17.5
	Hexanol	HEXO	102.18	157.55	8.0	10.7	1.8	13.3
	Heptanol	HEPO	116.16	176.45	0.1	NA	2.4	11.75
	Octanol	OCTO	130.23	195.16	0.059	10.3	2.9	10.34
	2-Ethyl Hexanol	ETHO	130.22	184.62	0.07	9.5	NA	4.41
	Decanol	DECO	158.23	231.05	*insoluble*	NA	4.0	5.82
ALDEHYDE	Butyl Acetate	BACA	116.16	126.05	0.43	8.5	1.7	5.01
	Pentyl Acetate	PACA	130.18	196	0.17	8.1	2.1	10
KETONE	Methyl Isobutyl Ketone	MIBK	100.16	119	1.693	9.4	NA	12.73
ALIPHATIC	Hexane	HEXA	86.16	68.73	0.00095	7.3	3.5	1.89
	Heptane	HEPT	100.16	98.45	*insoluble*	7.4	4.0	1.92
	Isooctane	ISOO	114.22	106.86	*insoluble*	6.9	4.5	1.94
	Cyclohexane	CYCL	84.16	80.73	0.01	8.2	3.2	2.02
OTHERS	Kerosene	KERO	NA	120 – 240	0.007 (23°C)	NA	NA	2.0 – 2.2
	Paraffin Liquid	PARA	NA		NA	NA	NA	NA

* NA = Not Available

Aliphatic hydrocarbon solvents exhibit the lowest degree of antibiotic partitioning with low solubility parameter and dielectric constant, and high *log P*, compared to solvent groups of alcohol, aldehyde and ketone. The trend of solvent polarity for this solvent group also exhibits a significant influence of molecular weight. A decrease in molecular weight contributes to increasing solubility parameter and decreasing *log P*. However it is found that the values of dielectric constant are not much different from hexane to cyclohexane (Table 1). In general, aliphatic hydrocarbon solvents show appreciably lower

partitioning efficiency compared with alcohol solvents especially for the partition of kitasamycin as shown in Figure 2.

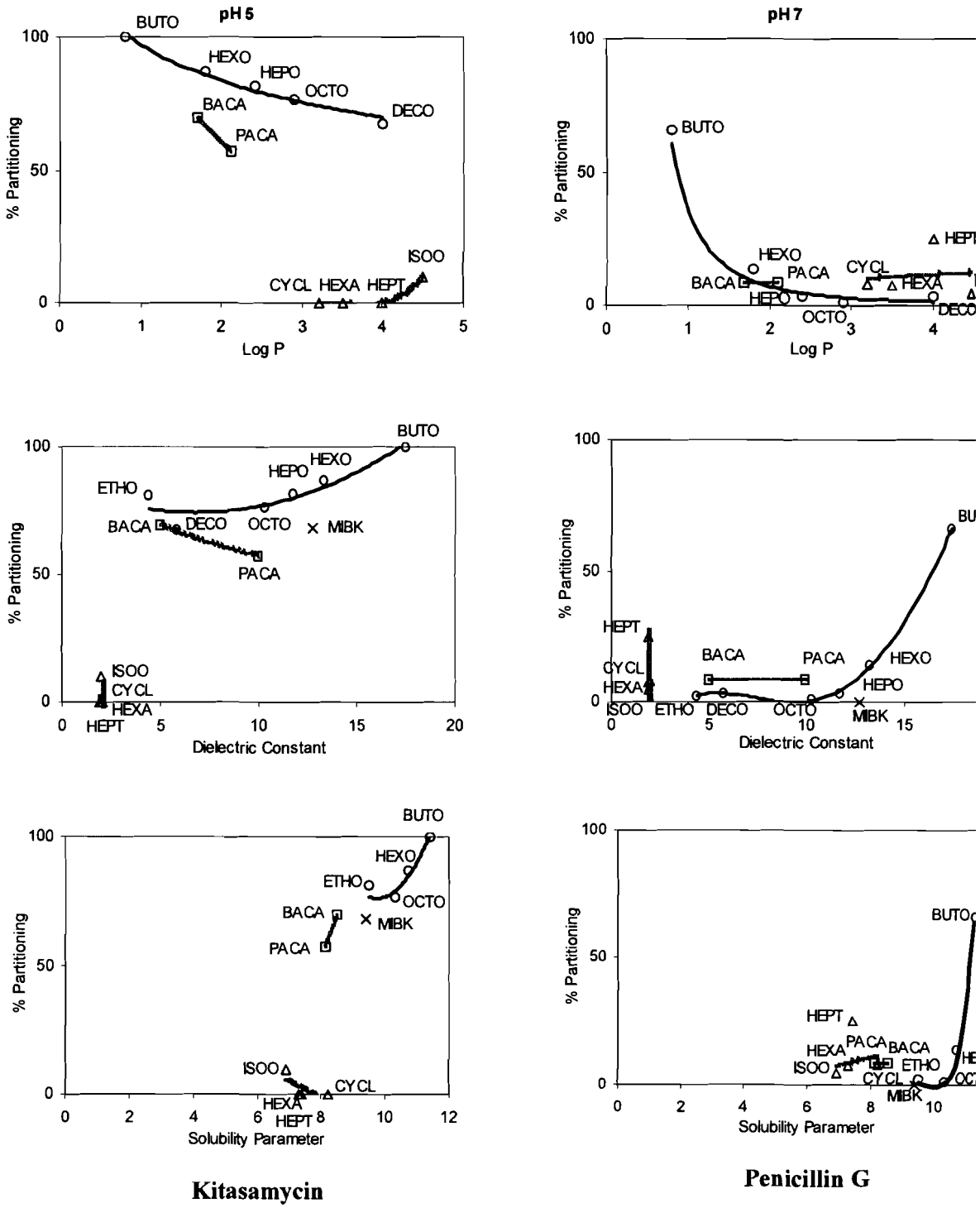


Figure 2 Effect of solvent polarity on the extent of kitasamycin partitioning at pH 5, and penicillin G partitioning at pH 7.

3.2 Effect of pH

From literature, pH is always indicated as a critical parameter for solute distribution between phases. An overview of the effect of pH on the partitioning behaviour of kitasamycin, penicillin G, rifampicin and teicoplanin using various types of solvents is given in Figure 3. As is evident from Figure 3, the partition of kitasamycin in organic solvents increases with an increase of pH up to a pH of 7, and slightly decreases at pH 8 for alcohol solvents. However, the degree of kitasamycin partitioning in butanol is generally high over the range of pH where it is 100% partitioned into butanol at pH 5. Meanwhile the variation of kitasamycin partitioning behaviour in decanol is very significant over the range of pH studied in this work, from the lowest degree of 17.9% at pH 3 up to the highest, 97.9% at pH 8. Solvent groups of aldehyde and ketone also exhibit a very high degree of kitasamycin partitioning at higher pH (pH 6-8). Meanwhile aliphatic hydrocarbons, kerosene and paraffin liquid show little or no partition of kitasamycin at all at low pH and slightly improved partitioning at higher pH.

However, if we look at the partitioning behaviour of rifampicin in alcohols and aldehydes (Figure 3), the effect of pH is not very significant but both alcohols and aldehydes show a generally high degree of rifampicin partitioning over the pH range of 3-8. The extent of partitioning with ketone, aliphatic hydrocarbons, kerosene and paraffin liquid on the other hand shows a very different dependence on pH where the partition of rifampicin decreases with an increase of pH up to a pH of 6-7 and slightly increases at pH 7-8.

The effect of pH on the partition of penicillin G and teicoplanin in organic solvents is quite inconsistent. For penicillin G, Figure 3 shows that the degree of partitioning increases from pH 3 to pH 5, and then it starts to descend from pH 5 to pH 8. This applies to almost every solvent group except for decanol and isooctane which exhibit an unpredictable dependence on pH. Meanwhile for teicoplanin, apart from butanol which experiences an obvious pH enhancement of antibiotic partitioning from pH 5-8, the partition of teicoplanin in other solvents is generally low from pH 3-7 except for a considerable increase of partitioning at pH 8 for hexanol and decanol but the overall degree of partitioning in these solvents is below 50%.

3.3 Effect of antibiotic types

Physico-chemical properties of the antibiotics used in this work are summarised in Table 2. From our experimental observation (Figure 4), kitasamycin and rifampicin display a very high degree of partitioning especially for solvent groups of alcohol and aldehyde over the pH range. Kitasamycin has a pK_a value of 6.75 and it exists in cationic form at pH below its pK_a; whilst rifampicin has two pK_a values, which are 1.7 and 7.9 and it can exist in cations and anions between the respective pHs [8,9]. In the pH range under study, using alcohols and aldehydes as solvents, which are due to their high solvent polarity, significantly enhances the partitioning of these antibiotics. For aliphatic hydrocarbon solvents (isooctane) and kerosene however, kitasamycin and rifampicin show reduced degrees of partitioning, not exceeding 40%, except for enhanced partition of rifampicin at pH 3 but still below 70%.

Meanwhile penicillin G and teicoplanin, in general, show poorer partitioning behaviour compared to kitasamycin and rifampicin. Penicillin G is a weak acid (pK_a = 2.75) and thus its physical extraction can be carried out most successfully at pH below its pK_a although the lipophilic character of penicillin G causes minor partitioning in organic solvents occurred over pH 3-8. Teicoplain is amphoteric (exists in cations, anions or zwitterions depending on the aqueous phase pH) and thus it is not soluble in non-polar solvents in general. Among all the solvents used, only butanol (with high solvent polarity) works better with penicillin G and teicoplanin with the extent of partitioning reaches as high as 70% and above. Our experiments on other solvents show either no partition or very low partition of these antibiotics, not exceeding 50%.

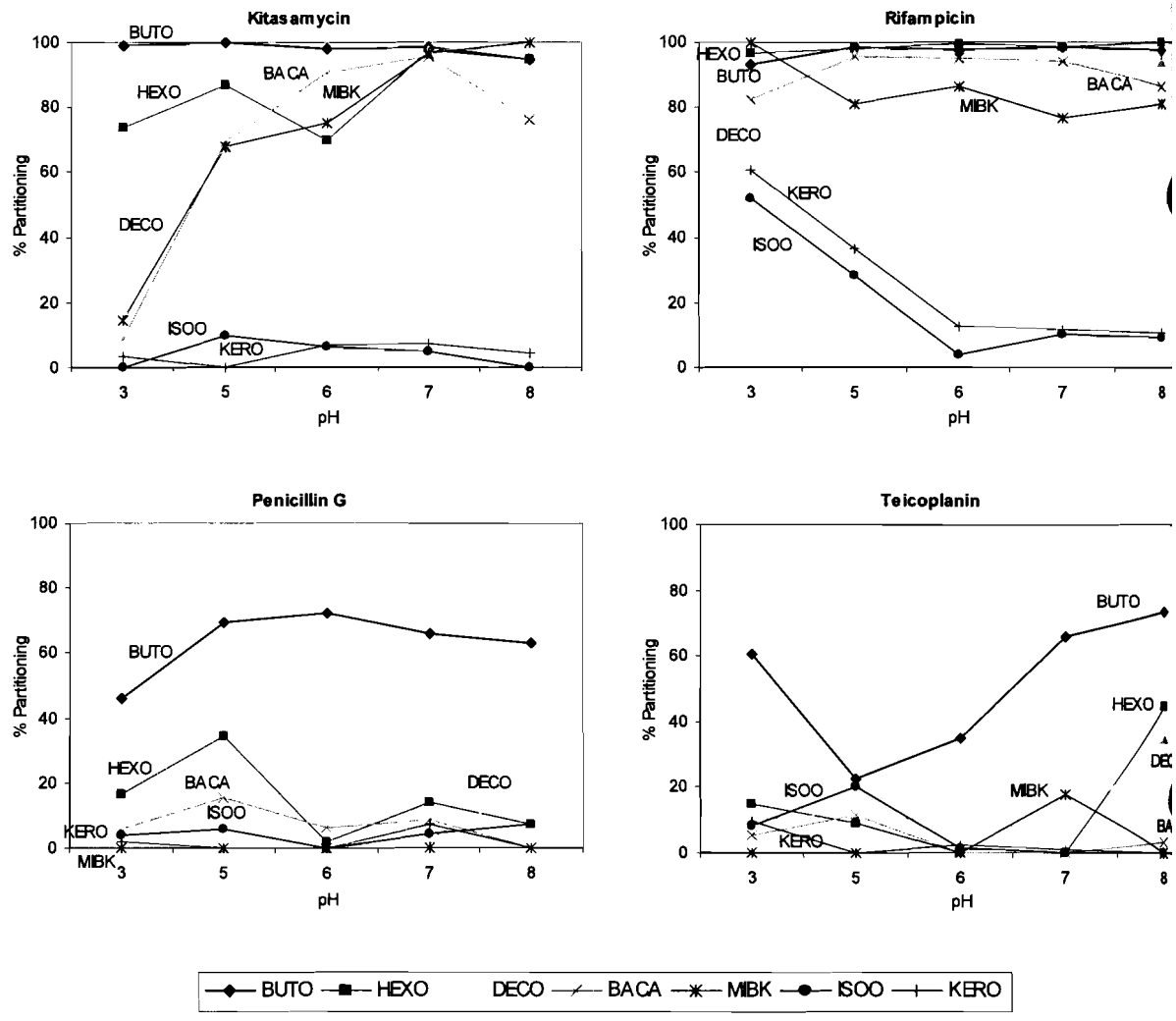


Figure 3 Effect of pH on the partitioning behaviour of kitasamycin, penicillin G, rifampicin and teicoplanin using various types of solvents.

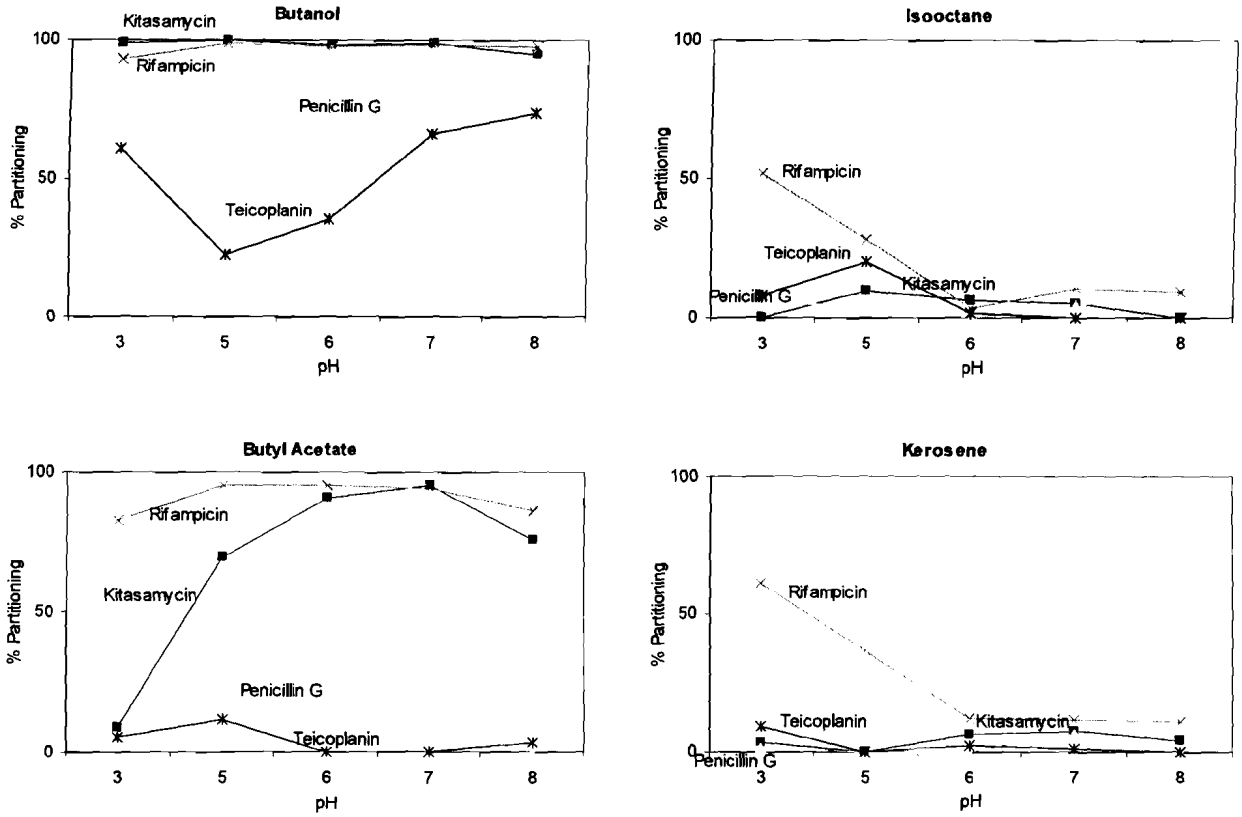


Figure 4 Effect of antibiotic types on the extent of partitioning using butanol, butyl acetate, isooctane and kerosene as solvents.

Table 2 Physico-chemical properties of the antibiotics used.

Antibiotic	**Teicoplanin** [b]	**Rifampicin**	**Kitasamycin**	**Penicillin G**
Antibiotic group [a]	Glycopeptides	Ansamycins	Macrolides	β-Lactams
Produced by	*Actinoplanes teicomyceticus*	*Norcardia mediterranei*	*Streptomyces kitasatoensis*	*Penicillium chrysogenum*
Other name	-	-	Leucomycin	Benzylpenicil
Molecular weight	1877	823	785	334
pK_a [c]	2.5, 9.2	1.7, 7.9	6.75	2.75
p*I*	4.2, 6.5	-	-	-
Solubility	Hydrophilic with a "hydrophobic tail" that aggregates to form micelles	Slightly soluble in water, acetone, ethanol (96%) and ether; freely soluble in chloroform and soluble in methanol	Lipophilic	Lipophilic

[a] Information taken from (*8,9*).

[b] Teicoplanin exists as a mixture of five similar compounds that differ by the number of carbons and substituent groups on the fatty acid side chain attached to the amino sugar.

[c] The pK_a is given for basic (cation forming) and acidic (anion forming) functional groups. In some instances more than one pK_a value is shown, indicating the presence of more than one ionisable group.

3.4 Effect of extractant addition

Figure 5 shows the results of antibiotic partitioning with addition of various extractants in the organic solvent, kerosene. It can be observed that the highest degree of partitioning (some up to 100%) occurred for teicoplanin, rifampicin and penicillin G using the extractant, TOMAC in kerosene. Though kitasamycin showed a very poor partitioning efficiency with TOMAC, it was found to have potential partitioning up to 73.29% with AOT. In general, TOMAC displayed excellent partitioning efficiency for all the antibiotics used except kitasamycin whereas AOT was the next superb extractant for all

except penicillin G. Other extractants such as DOA, TOA, AMBERLITE, TBP and DEPHA showed either greater or lesser partitioning efficiency than the one with kerosene only. Addition of these extractants did not significantly enhance the degree of partitioning compared with TOMAC and AOT. All antibiotics were poorly partitioned into pure kerosene system except for rifampicin which showed a moderate degree of partitioning up to the highest 60.91% at pH 3.

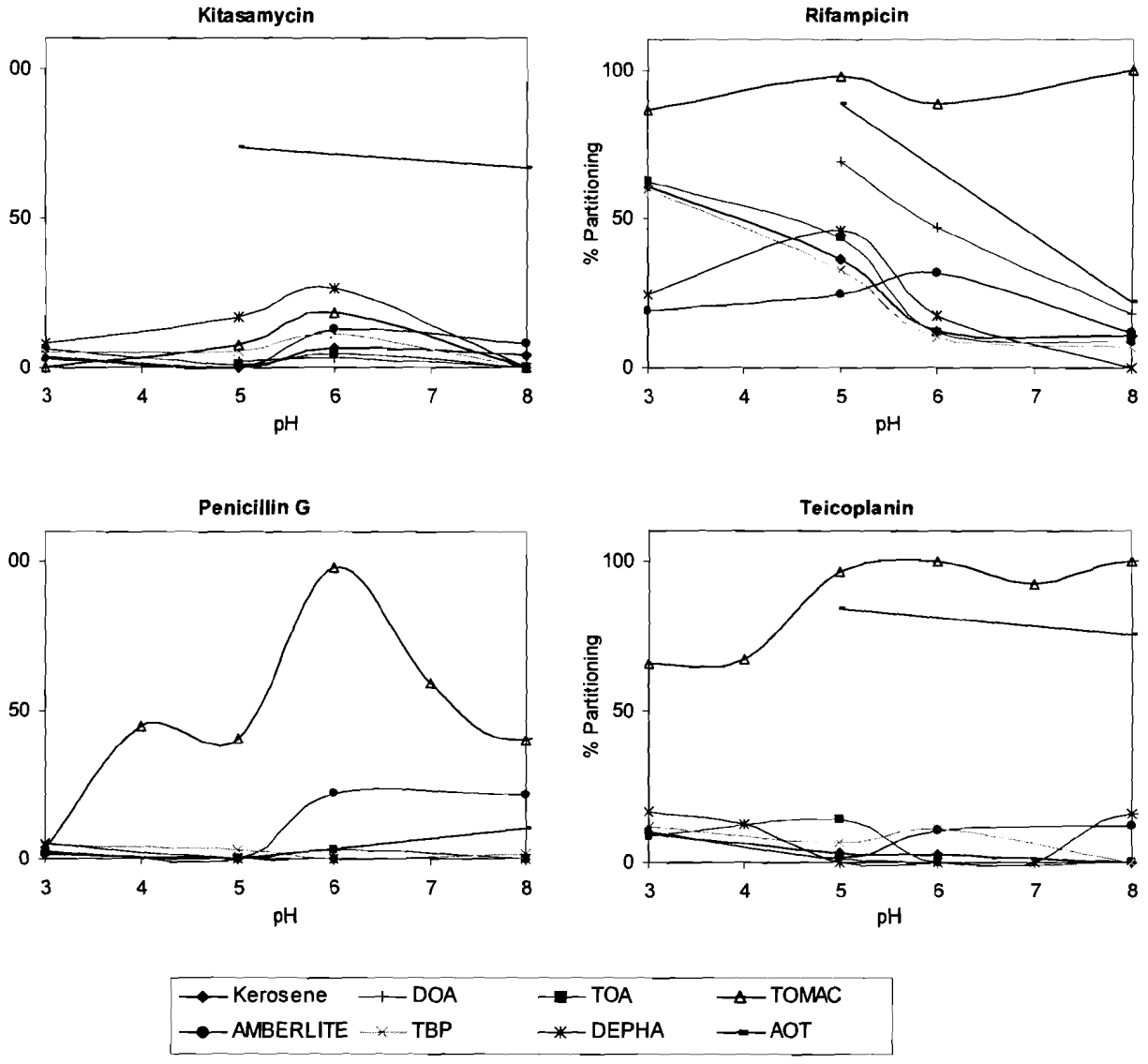


Figure 5 Effect of extractant addition on the partitioning behaviour of antibiotics in kerosene system.

TOMAC is a well-known liquid anion exchanger and therefore the anionic form of antibiotics is amenable for ion exchange reaction with TOMAC. In this case, the partition of antibiotics into TOMAC/kerosene system undergoes an ion exchange mechanism known as reactive extraction as follows:

$$Q^+Cl^- (o) + A^- (a) \Leftrightarrow Q^+A^- (o) + Cl^- (a)$$

where the removal of the anion A^- from the aqueous phase (a) occurs by ion exchange with the anion Cl^- of the ammonium salt Q^+Cl^- dissolved in the organic phase (o), forming an organic-soluble salt, Q^+A^-. It can be seen from Figure 5 that the degrees of partitioning for penicillin G, rifampicin and teicoplanin in TOMAC/kerosene system increase with increasing pH values from 3 to 8. There is a decrease in the partitioning efficiency after the optimum pH (i.e. pH 6) for penicillin G as a result of probable decomposition of penicillin G at high pH (*10*). At pH > acidic pK_a (Table 2), penicillin G (with acidic nature), rifampicin and teicoplanin (both with zwitterionic nature) dissociate in aqueous solution to form the anions and protons. The anionic form of these antibiotics thus reacts with TOMAC through ion exchange and forms an organic-soluble salt, which causes the degree of partitioning into the organic solution increase.

On the other hand, the contact of antibiotics with anionic AOT most probably leads to the formation of water in oil microemulsions (known as reverse micelle extraction where AOT is termed as surfactant in this case). Only cationic form of antibiotics would partition in this AOT/kerosene system. Figure 5 shows a considerable degree of partitioning with AOT for kitasamycin, rifampicin and teicoplanin especially at pH 5. Since the partition of these antibiotics with anionic AOT occurs at pH where it is positively charged (pH < basic pK_a), it is believed that electrostatic attractions between the antibiotic and AOT play a significant role in the partitioning process where reverse micelle extraction might take place. Other partitioning mechanisms with secondary and tertiary amines as well as P-O extractants are less significant and thus would not be further discussed in this study.

4.0 CONCLUSIONS

As a whole, the partitioning behaviour of antibiotics in organic solvents is very much influenced by solvent polarity, pH, antibiotic types and addition of extractant. Higher solvent polarity contributes to higher partitioning of antibiotics. The polarity of solvents, in general, increases with increasing solubility parameter and dielectric constant; and with decreasing log P. Alcohols are the most favourable solvent group for antibiotic partitioning despite their high mutual solubility of the phases. Aldehydes and ketones rank the second notwithstanding their high cost. Hydrocarbon solvents interact with antibiotics only slightly. With addition of extractants in kerosene, different partitioning mechanisms could be operative depending on the types of extractants used. According to the pK_a values, anionic form of antibiotics such as penicillin G can partition into TOMAC/kerosene system by ion exchange reaction while cationic form of antibiotics such as kitasamycin can partition into AOT/kerosene system by reverse micelle extraction. Zwitterionic antibiotics like teicoplanin and rifampicin undergo both mechanisms corresponding to their pK_a values. The effect of pH is very important as a controlling factor in the partitioning behaviour of antibiotics in this study. By selective screening of types of solvents and extractants and manipulating the optimum partitioning pH, the most favourable solute transfer between aqueous and organic phases can be obtained for different types of antibiotic. This study will be useful for further investigation and development in the use of solvent extraction for antibiotic separation.

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