

## Introduction

Extraction is a versatile technique used, among other things, to study the concepts of acid-base and polarity through an extraction procedure with 2,6-dichlorophenolindophenol (DCPIP).

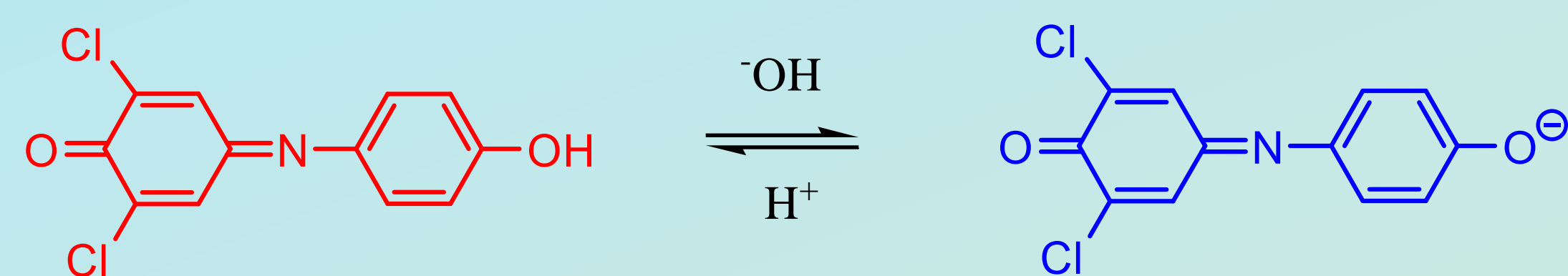


Figure 1: Chemical structure of 2,6-dichlorophenolindophenol and colour change as a function of pH

The dye is used to produce a sol-gel that can measure iron (III) ions in an aqueous medium. The sol-gel can then be applied to a glass slide and measured using a spectrophotometer. Iron ions then form a 1:1 complex with the dye (present in the sol-gel) giving rise to a change in absorbance due to the strong colour change. This technique can then be used to determine the iron concentration in unknown samples.<sup>[1]</sup>

A physical mixture of a drug and another component is separated by extraction based on polarity differences, and the purity of the components is evaluated using their melting temperature ( $T_m$ ).

The use of DCPIP and the separation of the physical mixture can serve as didactic material, providing first-year students with insights into the acid-base properties and polarity of drugs.

Partition coefficients ( $\log P$ ) of drugs are determined and compared with the values reported in the literature. Drugs require an appropriate polarity to ensure effective bioavailability. They must dissolve in the aqueous matrix of the gastrointestinal tract while maintaining sufficient lipophilicity to permeate the phospholipid bilayer and enter the bloodstream. Figure 2 shows the formula used to determine the  $\log P$ . It is ideally between 0 and 5 for candidate drugs under investigation.<sup>[2]</sup>

$$\log P = \log \left( \frac{C_{1-octanol}}{C_{water}} \right)$$

Figure 2: Formula for the  $\log P$  of a drug

## Materials and methods

NaOH, HCl, benzocaine, paracetamol, aspirin, and salicylic acid were purchased from Sigma-Aldrich. DCPIP, dichloromethane (DCM) and nitrofurantoin were purchased from Acros Organics. For partition coefficient determination, 1-octanol was purchased from Sigma-Aldrich. The devices used included a rotary evaporator, melting point apparatus, and Fourier Transform Infrared (FT-IR) spectrometer (Spinsolve Education 43 MHz). Thin-Layer Chromatography (TLC) was employed to evaluate separations.

## Results and discussion

The transitions between the acidic and basic forms of the dye are shown in Figure 1.<sup>[3]</sup> In separating funnel A ( $SF_A$ ), the dye (DCPIP) was dissolved in the aqueous phase. Since the dye occurs in its anionic form ( $A^-$ ), it remains in the polar aqueous phase and does not migrate to the non-polar dichloromethane (DCM) layer.

In  $SF_B$ , hydrochloric acid (HCl) was added to the aqueous phase. This results in the protonation of the anionic  $A^-$  to the neutral form (HA), which exhibits a red colour (see Figure 1). Since HA is more soluble in the non-polar DCM layer, it partially migrates into the DCM phase during vigorous shaking, as seen in  $SF_C$ . This causes the DCM layer to turn red, while the aqueous phase retains only a faint red hue due to the presence of trace HA.

In  $SF_D$ , sodium hydroxide (NaOH) was added to the aqueous top layer. This partially neutralises HA to the anionic form  $A^-$ , which exhibits a blue colour. However, upon gentle mixing, part of the neutral form HA remains in the DCM layer, resulting in a blue-coloured aqueous phase and a red-coloured DCM layer. After vigorous shaking in separating funnel E, the entire amount of HA migrates to the aqueous phase, where it is completely converted to the anionic form  $A^-$  by reaction with NaOH.

This leads to a completely blue aqueous top layer and a colourless DCM layer, making the situation identical to that in  $SF_A$ .



Figure 3: Colour change and phase transition as a function of change in pH and polarity

Physical powder mixtures, each containing a drug as one component, were separated by extraction. The yield (% rec.) after separation and the solvent used to dissolve the powder mixture prior to extraction are shown in Table 1. The recovery yields were acceptable. Purity was confirmed by comparing the experimental melting point ( $T_{m,exp}$ ) of the separated compound with the reference value ( $T_{m,ref}$ ), and by Infrared Spectroscopy (see Figure 4). All the results are summarized in Table 1.

Table 1: Summary table for results of separation of 2 components

Organic solvent	Component 1				Component 2			
	Name	% rec.	$T_{m,ref}$ (°C)	$T_{m,exp}$ (°C)	Name	% rec.	$T_{m,ref}$ (°C)	$T_{m,exp}$ (°C)
1 Dichloromethane	Aspirin	40	136,3	136,4	Fluorene	96	116,2	115,8
2 Dichloromethane	Benzocaine	43	90,2	90,1	4-MAF*	71	39,8	38,4
3 2-Me THF*	Salicylic acid	47	162,9	161,4	Fluorene	85	116,2	117,3
4 Dichloromethane	Salicylic acid	53	162,9	163,3	4-MAF	84	39,8	39,8

\*2-Me THF = 2-methyltetrahydrofuran, 4-MAF = 4-methoxyacetophenone

Separation 4 (Table 1) is further explained. In the IR spectrum of salicylic acid, the  $\nu_{C=O}$  band is observed at  $1655\text{ cm}^{-1}$ , while in the IR spectrum of 4-methoxyacetophenone, the  $\nu_{C=O}$  band is observed at  $1667\text{ cm}^{-1}$ . In the spectrum of the mixture, the characteristic bands of both individual components disappear, confirming the success of the separation. Specifically, the  $\nu_{C-H}$   $sp^3$  bands at  $2964\text{ cm}^{-1}$ , characteristic of 4-methoxyacetophenone, are no longer present in the spectrum of salicylic acid. Similarly, the  $\nu_{O-H}$  bands at  $3230\text{ cm}^{-1}$  and  $2590\text{ cm}^{-1}$ , as well as the  $\delta_{O-H}$  (Ar-OH) band at  $1207\text{ cm}^{-1}$ , characteristic of salicylic acid, are absent in the spectrum of 4-methoxyacetophenone. The band at  $2840\text{ cm}^{-1}$  in the spectrum of salicylic acid is attributed to an overtone and should not be mistaken for an  $sp^3$ -hybridized  $\nu_{C-H}$ .

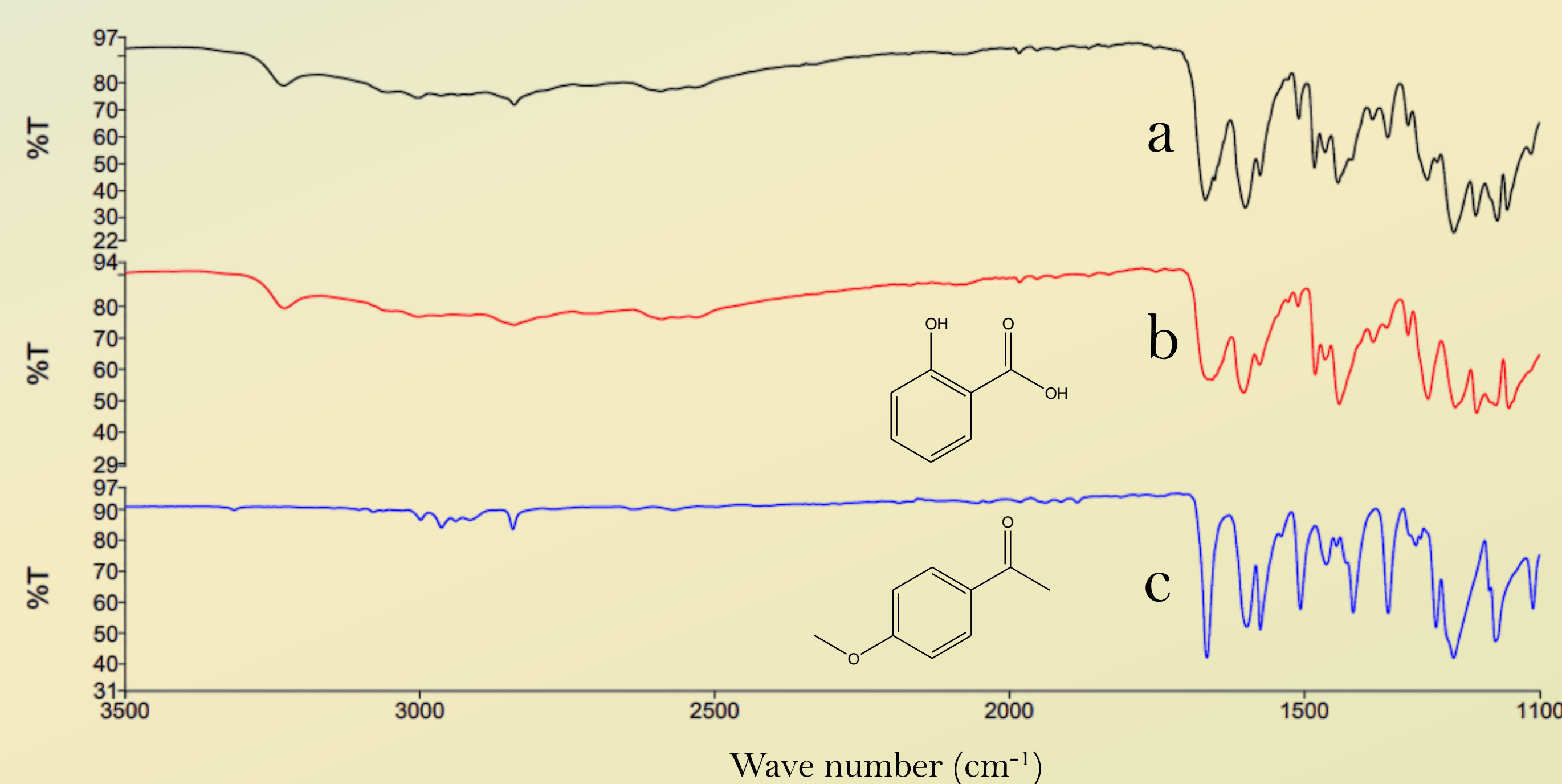


Figure 4: (a) IR spectrum of the physical mixture (salicylic acid and 4-MAF), (b) IR spectrum of salicylic acid after separation, (c) IR spectrum of 4-methoxyacetophenone after separation

Finally,  $\log P$  values of various drugs were also determined. Unfortunately, it was not possible to remove 1-octanol using the rotary evaporator. Therefore, an alternative was used, employing simple distillation with an oil bath. After distillation, the mass of the drug was determined so that the  $\log P$  could be determined (see Figure 2). literature values. This can be explained by different methods used in the literature to determine the  $\log P$ .<sup>[4]</sup>

Table 2 shows all the results.  $\log P_{lit}$  is the literature value and  $\log P_{exp}$  is the experimental value. The results do not fully align with the literature values. The method can be optimised to achieve better agreement with the literature values.

Table 2: Comparison of experimental and literature  $\log P$  values for drugs

Drug	$\log P_{lit}$	$\log P_{exp}$
Paracetamol	0,46	0,37
Nitrofurantoin	0,68	0,69
Aspirin	1,19	0,87
Salicylic acid	2,26	1,90

## Conclusion

DCPIP is a suitable dye for introducing and explaining acid/base properties and polarity, which is particularly valuable for first-year students. Besides DCPIP, there are alternative dyes, such as Congo red, which can also be used in this didactic framework.

Separation of the two components proceeds successfully and serves as a further illustration of acid/base properties for students. An additional challenge is that students need to track where the individual components are after adding an acid or base. In addition, the selection and optimisation of suitable solvents improves efficiency, as it is often found that one of the two components does not dissolve in a particular solvent. The use of green solvents also contributes to the sustainability of the study.

Finally, the procedure for determining the  $\log P$  value optimises both the yield and accuracy of the measurements. In addition, the study expands by including more drugs in the analysis.

## References

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