Physico-Chemical Characterization of Drugs: Acidity and Solubility

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PHYSICO-CHEMICAL CHARACTERIZATION OF DRUGS: ACIDITY AND SOLUBILITY

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This project has been presented by **Elham Shoghi Kalkhoran** to obtain **PhD** at **University of Barcelona**

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APPROVE

That this present PhD thesis presented by Elham Shoghi Kalkhoran has been done under their supervision at Analytical Chemistry Department at University of Barcelona.

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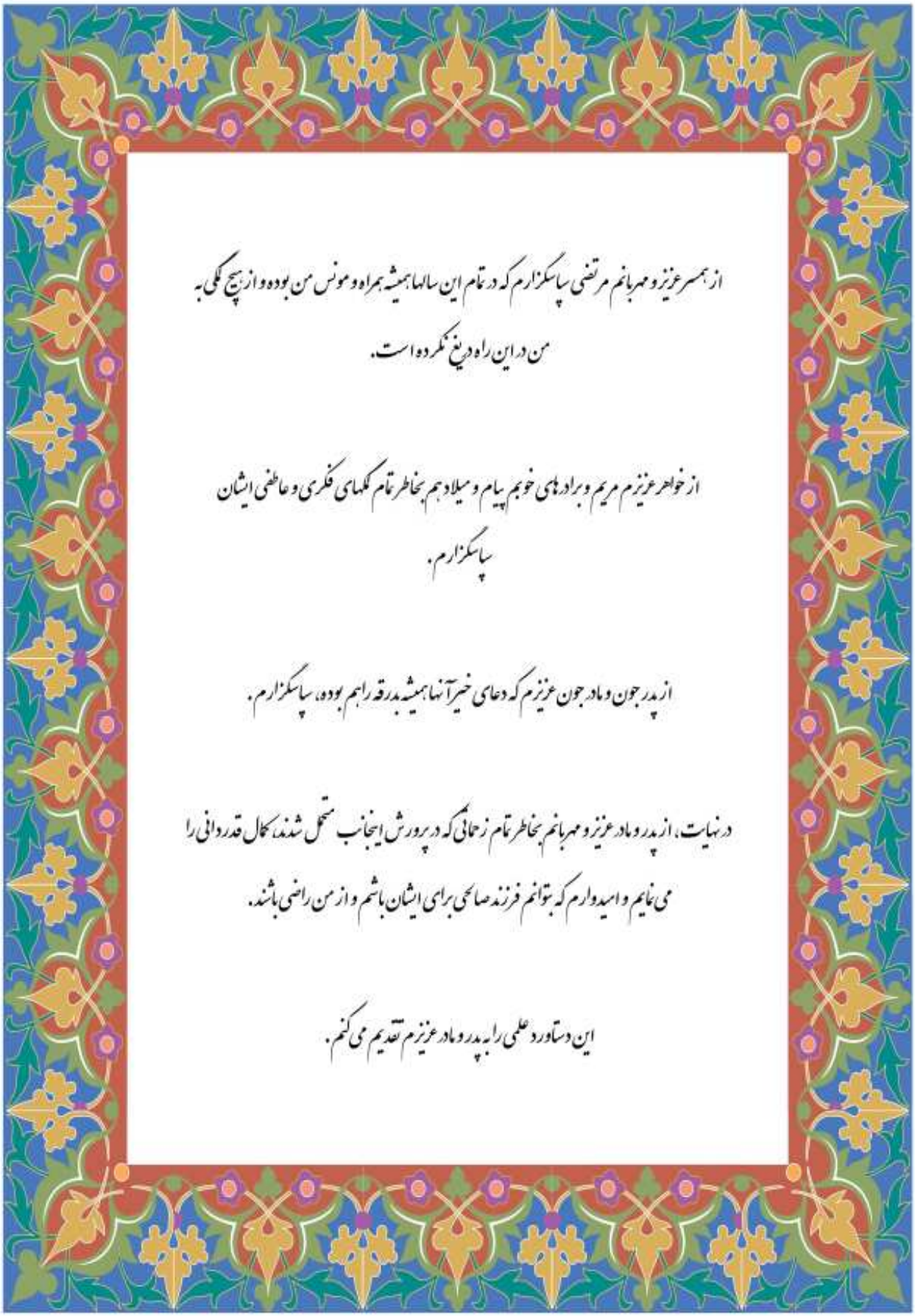

از همسر عزیز و مهربانم مرتضی سپاسگزارم که در تمام این سالها همیشه همراه و مونس من بوده و از هیچ کمکی به من در این راه دریغ نکرده است.

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Contents

PRÓLOGO

1. OBJETO

El objeto de la presente Tesis es explorar las posibilidades, establecer los límites y validar la metodología potenciométrica establecida para la determinación de dos propiedades fundamentales en las primeras etapas de desarrollo de los principios activos farmacéuticos, etapas conocidas en su conjunto como *Drug Discovery*. Estas propiedades son la acidez y la solubilidad y son determinantes para la inclusión de las moléculas en la clasificación biofarmacéutica, *Biopharmaceutical Clasification System* (BCS). Las propiedades físico-químicas seleccionadas para este trabajo no son las únicas a tener en cuenta pero, junto con la lipofilicidad, constituyen el grupo más significativo de parámetros determinables en el laboratorio para estimar la futura biodisponibilidad de las moléculas emergentes.

Hoy día se dispone de metodología potenciométrica relativamente rápida y de precisión para la caracterización físico-química de sustancias ácidas o básicas. Sin embargo, resulta necesaria una revisión de las técnicas en uso y de sus límites de aplicabilidad ya que las nuevas sustancias emergentes son moléculas de complejidad creciente y suponen nuevos retos, por ejemplo, solubilidades muy altas o muy bajas, sustancias con tendencia a formar agregados moleculares o iónicos, etc. El trabajo planteado se orienta a la validación crítica de la metodología mencionada habida cuenta las necesidades que surgen continuamente en los laboratorios farmacéuticos.
Otro aspecto de interés relacionado con la biodisponibilidad de los principios activos es su velocidad de disolución. También ha sido objeto de este trabajo estudiar algunos aspectos relacionados con la preparación farmacéutica de un principio activo muy insoluble para incrementar su velocidad de disolución. Este aspecto del trabajo planteado debe abrir la puerta a la utilización más eficiente de fármacos de solubilidad reducida en el tracto intestinal.

2. RESUMEN

El objetivo del presente trabajo ha sido contribuir a establecer metodología robusta y de *highthroughput* de interés en la etapa conocida como "*Drug Discovery*" que tiene lugar en los laboratorios farmacéuticos al inicio del proceso de desarrollo de nuevos fármacos. Este objetivo ha implicado la exploración de las posibilidades de la metodología potenciométrica establecida y comercializada por Sirius Analytical Ltd. para la determinación de las constantes de acidez y de la solubilidad de compuestos bioactivos y también un estudio sobre la mejora de la biodisponibilidad de un fármaco muy insoluble tomado como modelo mediante el aumento de su velocidad de disolución.

En la primera parte de esta Tesis, se han determinado potenciométricamente las constantes de disociación ácida y la variación de entalpía asociada de dos bases y dos ácidos tomados como modelo en agua pura y en mezclas de metanol/agua (0-60% w/w) a varias temperaturas (25-55^{o}C). Esto ha implicado la puesta a punto de la estandarización del sistema potenciométrico en las condiciones de trabajo. Los valores de pK_a determinados son concordantes con los que ofrece la literatura. Se han calculado también las entalpías de disociación en los distintos solventes binarios estudiados mediante la ecuación de Van't Hoff a partir de los valores experimentales de pK_a. La consistencia de los resultados obtenidos con los de la literatura, obtenidos directamente por calorimetría, confirma la robustez de la metodología.

En la segunda parte de este trabajo, el estudio se centró sobre la determinación potenciométrica de la solubilidad de ácidos y bases mediante el método conocido como Chasing Equilibrium, como alternativa a los procedimientos clásicos de equilibración. El método es rápido y produce resultados precisos. Se ha realizado un estudio sobre las condiciones experimentales óptimas en términos de peso de la muestra para medir eficazmente la solubilidad. El estudio muestra que, en función de la naturaleza y solubilidad de los compuestos, existe un intervalo limitado de peso de muestra adecuado para obtener resultados fiables.

En la tercera parte de la presente memoria, se estudian los perfiles de solubilidad en función del *pH* de cinco fármacos ionizables de naturaleza diferente, un ácido y una base monopróticos, una base diprótica y dos compuestos anfóteros que muestran una especie zwitteriónica cada uno. Se han determinado los perfiles de solubilidad mediante el método clásico de equilibración (Shake-Flak, S-F) y el potenciométrico y, en ambos casos, se han utilizado las relaciones apropiadas de Henderson-Hasselbalch (H-H) o derivadas. Los resultados obtenidos de forma independiente por ambos métodos son consistentes. Se ha hecho un estudio crítico acerca de la influencia del electrolito utilizado como agente tampón en el método S-F en los valores de solubilidad obtenidos y se han observado algunas desviaciones de los puntos experimentales con respecto a los perfiles esperados que pueden ser debidas a interacciones específicas entre el electrolito tampón y el fármaco. En otros casos, las desviaciones observadas son independientes de los tampones utilizados y se pueden atribuir a la formación de nuevas especies tales como agregados iónicos del fármaco en estudio o la precipitación de una sal a partir de una especie catiónica del compuesto analizado.

En la cuarta parte de esta memoria el objetivo ha sido estudiar la velocidad de disolución de comprimidos preparados a partir de dispersiones sólidas de un fármaco modelo con y sin portador del fármaco y también en presencia y en ausencia de tensioactivo en soluciones acuosas neutras y ácidas. Como fármaco modelo se estudió la Anfotericina B y se utilizaron como portadores manitol e inulina y como tensioactivos se ensayaron el deoxicolato de sodio (SDC) y el laurilsulfato de sodio (SLS). La difracción de rayos X reveló que el fármaco en estudio se hallaba en estado amorfo en todas las dispersiones sólidas estudiadas. Se puede concluir que la velocidad de disolución del fármaco se incrementa significativamente en presencia de portador y tensioactivo.

3. PUBLICACIONES

Hasta el momento los resultados obtenidos en esta Tesis se han dado a conocer mediante las siguientes publicaciones.

- **Enthalpies and constants of dissociation of several neutral and cationic acids in aqueous metanol/wáter solutions at various temperatures**
  E. Shoghi, L. Romero, M. Reta, C. Ràfols, E. Bosch
  Journal of Pharmaceutical Sciences, 49, (2009), 923-930.

- **Kinetic and thermodynamic solubility values of some bioactive compounds**
  E. Shoghi, E. Fuguet, C. Ràfols, E. Bosch
  Chemistry and Biodiversity, 6, (2009), 1789-1795.

- **Solubility-*pH* profiles of ionisable compounds. Effect of buffer nature and ionic strength on solubility values**
  E. Shoghi, E. Fuguet, E. Bosch, C. Ràfols
  European Journal of Pharmaceutical Sciences, DOI: 10.1016/j.ejps.2012.10.028, (en prensa).

INTRODUCCIÓN GENERAL

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La presión creciente que sufren las empresas farmacéuticas para acelerar el descubrimiento y desarrollo de nuevos fármacos y para reducir los costes asociados, empuja a los laboratorios a establecer metodología eficiente para la caracterización de los productos emergentes. La bibliografía muestra diferentes estrategias para abordar el problema basadas en cálculos *in silico* o en medidas experimentales *in vitro* (1-3). El objetivo final es conseguir un procedimiento eficaz para la evaluación de propiedades tales como la absorción, distribución, metabolismo, excreción y actividad tóxica (ADMET) de compuestos con previsibles aplicaciones farmacológicas. En la práctica, resulta del mayor interés disponer de una estimación rápida y fiable de los parámetros críticos para tomar decisiones bien fundamentadas y conducentes a descartar de manera muy temprana los candidatos a fármaco con propiedades inadecuadas. El objetivo es detectar rápidamente aquellas moléculas con alta actividad *in vitro*, selectividad adecuada, características físico-químicas dentro de los intervalos prefijados, farmacocinética aceptable y toxicidad mínima. De hecho, en el pasado, se ignoraban muy a menudo valores poco adecuados de las propiedades físico-químicas de las nuevas moléculas y sus efectos negativos se descubrían en las etapas finales del desarrollo de éstas con el consiguiente impacto negativo sobre el éxito clínico y sobre los costes generales.

La acidez, la solubilidad y la lipofilicidad están entre las propiedades fisicoquímicas más importantes a examinar para un nuevo candidato a fármaco y son parámetros fundamentales para la estimación de las propiedades ADMET de nuevas moléculas (1, 4). Su evaluación temprana durante la etapa inicial de desarrollo, conocida como *drug discovery*, proporciona una información muy interesante que permite interpretar mejor los resultados del cribado y contribuir de manera eficaz al diseño de nuevas moléculas (5). A pesar de ello, el hecho de obtener valores asociados a estas propiedades físico-químicas poco adecuados no debe ser la única razón para rechazar un compuesto prometedor que ofrezca *in vitro*, por ejemplo, gran afinidad y selectividad respecto al receptor, aunque hay que asumir el riesgo de un mal resultado en etapas avanzadas de su desarrollo.

El uso de métodos *in silico* y de mediciones experimentales rápidas y de calidad (*highthrougput*) es importante en el proceso de generación de nuevos compuestos, en particular cuando se planifican nuevas síntesis para crear una librería de compuestos o cuando se dispone de una cantidad limitada de material. En cualquier caso, la creciente popularidad de la estimación *in silico* de la constante de acidez, la solubilidad y la lipofilicidad no puede sustituir totalmente a las mediciones experimentales de estas propiedades y, en el caso de desarrollo de nuevos fármacos, resulta imprescindible la determinación experimental de las propiedades mencionadas en la etapa inicial de su desarrollo. Hay que señalar aquí que los datos experimentales de alta calidad obtenidos utilizando métodos fiables son especialmente valiosos cuando de trata de evaluar una nueva clase de compuestos. La importancia de las propiedades físico-químicas en el diseño de fármacos ha sido ampliamente reconocida y, de hecho, algunos procesos integrados para la medición de las propiedades fisico-químicas de los candidatos a fármaco se encuentran hoy rutinariamente incorporados en las etapas de *drug discovery* en muchas compañías farmacéuticas (6-12).

Uno de los objetivos principales de la industria farmacéutica es diseñar moléculas con actividad terapéutica y absorción suficiente. En consecuencia, se suele optar por un método iterativo para mejorar propiedades tales como la solubilidad, la lipofilicidad y la permeabilidad, todas ellas estrechamente relacionadas con la absorción del compuesto a través de las membranas biológicas. Sin embargo, los parámetros mencionados anteriormente son únicamente sustitutos físico-químicos de la absorción *in vivo*, relativamente fáciles de medir. Durante las últimas décadas se han propuesto diversos enfoques para predecir la absorción de las moléculas con presunta bioactividad a partir de sus parámetros físico-químicos. Entre estos cabe destacar el bien conocido "sistema de clasificación biofarmacéutica" (BCS), que se ha ido perfeccionando en los últimos años.

La hipótesis de que el efecto terapéutico de los fármacos depende de la concentración de fármaco en las proximidades de su diana constituye la base sobre la que se propuso la BCS. Hay que tener en cuenta que la absorción del fármaco en la circulación sistémica es un requisito previo de todos los medicamentos para alcanzar su diana,

excepto para aquellos que se aplican directamente en el lugar donde deben actuar, o los que se inyectan por vía intravenosa. En el caso más frecuente, el de la administración oral (ruta gastrointestinal) hay muchos factores que afectan a la biodisponibilidad (fracción de fármaco que alcanza la circulación sistémica). Dado que sólo el fármaco disuelto es capaz de atravesar la membrana gastrointestinal, la solubilidad es uno de esos factores. Sin embargo, el metabolismo del fármaco en la luz intestinal, la pared intestinal y el hígado puede reducir su biodisponibilidad. En general se puede afirmar que la velocidad de absorción, es decir el inicio y la extensión de los efectos clínicos, vienen determinados por la disolución del fármaco y el transporte posterior a través del intestino y del hígado. Estos dos aspectos constituyen la base de la BCS [13], la cual se encuentra actualmente incorporada a las directrices de la Administración de Alimentos y Medicamentos (FDA). De acuerdo con la BCS (Tabla 1) se pueden distinguir cuatro tipos diferentes de fármacos.

Tabla 1. Sistema de Clasificación Biofarmacéutica

Clase	Solubilidad	Permeabilidad
I	Alta	Alta
II	Baja	Alta
III	Alta	Baja
IV	Baja	Baja

Los dos parámetros directamente implicados en la BCS, son la solubilidad y la permeabilidad.

- Solubilidad: Una sustancia medicamentosa se considera muy soluble cuando la dosis de efecto máximo es soluble en 250 mL o menos de agua en un intervalo de *pH* de 1.0-7.5 a 37°C. La solubilidad es una propiedad muy importante, pero la efectividad de un fármaco depende también de su velocidad de disolución. De hecho, un fármaco se considera de disolución rápida cuando el 85% o más de la cantidad declarada de fármaco en un preparado farmacéutico se disuelve en un

tiempo máximo de 30 minutos en un volumen de 900 mL o menor de solución tampón, usando un aparato estandarizado USP 1 o 2 [14].

- Permeabilidad: Una sustancia medicamentosa se considera altamente permeable cuando el grado de absorción en seres humanos es mayor que el 90% de la dosis administrada calculada sobre la base del equilibrio de masas o en comparación con una dosis intravenosa de referencia. La permeabilidad está estrechamente relacionada con la lipofilicidad de la molécula, comúnmente expresada por su distribución entre n-octanol y agua, log $P_{o/w}$.

Resulta evidente que, en función de la clase de moléculas a examinar, deben aplicarse diferentes estrategias para aumentar o acelerar su absorción: o bien aumentando la permeabilidad de la membrana absorbente o bien aumentando la cantidad de fármaco disuelta.

- Fármacos de Clase I: presentan alta permeabilidad y alta solubilidad. La etapa limitante de su absorción es la velocidad de disolución del fármaco, y, si ésta es muy rápida, la velocidad de vaciado gástrico. Estos compuestos se absorben bien, y, en general, su velocidad de absorción es más alta que la de excreción.

- Fármacos de Clase II: muestran una permeabilidad alta y una baja solubilidad. En este caso, la disolución del fármaco *in vivo* es el paso limitante para la absorción excepto en el caso de dosis muy altas. En general, la absorción de los fármacos de Clase II es más lenta que para los de Clase I y se produce durante un período de tiempo más largo [14]. La estrategia para compuestos de Clase II consiste en aumentar la cantidad de moléculas de fármaco disuelto en el lugar de absorción [15-19], en el bien entendido que esta estrategia resulta útil siempre que la permeación no sea limitante. La posible limitación debida a la permeación depende principalmente del mecanismo de transporte a través de la membrana. Cuando el fármaco se transporta a través de la membrana mediante difusión pasiva, el flujo a través de la membrana aumenta proporcionalmente a la concentración de fármaco en el sitio de absorción. Sin embargo, cuando el transporte del fármaco se produce

mediante un portador, esto no es necesariamente así, ya que la capacidad de transporte del portador puede convertirse en limitante de la velocidad de absorción.

- Fármacos de Clase III: son los que tienen una solubilidad alta y una permeabilidad baja. En consecuencia, la permeación a través de la membrana es la etapa limitante de la velocidad de absorción. La estrategia a seguir para estos medicamentos consiste en aumentar la permeabilidad de la membrana absorbente y esto depende mucho del mecanismo de transporte a través de la misma. El tema ha despertado un gran interés en la química médica y, de hecho, existen numerosos estudios sobre la manera de aumentar la permeabilidad de membrana a lo largo del tracto gastro-intestinal [25-29].

- Fármacos de Clase IV: en este caso tanto la solubilidad como la permeabilidad son deficientes y, en consecuencia, son principios activos problemáticos para una administración oral eficaz ya que presentan una biodisponibilidad escasa. En general no se absorben bien a través de la mucosa intestinal, y cabe esperar una variabilidad notable en su comportamiento [14]. Para mejorar la absorción de un fármaco de Clase IV, es necesario aumentar tanto la solubilidad como la permeabilidad. Sin embargo, el aumento de la solubilidad resulta más eficaz que el aumento de la permeabilidad debido a que, en la práctica, la cantidad de fármaco disuelto en el sitio de absorción puede variar más de seis órdenes de magnitud (de 0,1 μg/L a 100 mg/L), mientras que la permeabilidad lo hace en un intervalo de solo 50 veces. En consecuencia, aumentar la absorción mediante el aumento de la concentración de fármaco ofrece un potencial mayor de mejora de la absorción y resulta más práctico incluso en el caso que la permeabilidad resulta comprometida [6].

Hay que hacer notar aquí que el sistema de clasificación BCS tiene por objeto la diferenciación de los fármacos administrados por vía oral, pero se puede aplicar también a su absorción después de ser administrados por otras vías. Por ejemplo, para obtener una absorción sistémica mediante la administración pulmonar, el fármaco tiene que disolverse en la mucosa de los alvéolos antes de que pueda ser transportado a través de la membrana alveolar.

Para aumentar la cantidad de fármaco disuelto en el sitio de absorción se pueden utilizar diversas estrategias. La estrategia para hacer frente a los fármacos de absorción limitada debido a su baja solubilidad (Clase II y Clase IV) es aumentar su velocidad de disolución. Sin embargo, la absorción de fármacos lipofílicos se frena, muy a menudo, por la lenta velocidad de disolución de las partículas sólidas de fármaco. En consecuencia, se trata de conseguir su dispersión en forma de partículas muy finas para aumentar así la superficie del sólido y, de acuerdo con la clásica ecuación de Noyes-Whitney, aumentar la velocidad de disolución [20]. Hay que señalar aquí que la reducción del tamaño de partícula puede llegar a la nano-escala. Sin embargo, incluso esta reducción de tamaño puede no conducir a concentraciones superiores a la solubilidad máxima del fármaco, es decir a soluciones sobresaturadas, en los fluidos intestinales. Alternativamente, para aumentar la velocidad de disolución de fármacos poco solubles se pueden utilizar las dispersiones sólidas, es decir, el fármaco presente en estado amorfo y/o en forma de cristales pequeños, incorporado a un portador o a una matriz adecuada [21-23]. Las dispersiones sólidas han demostrado gran eficacia para aumentar la cantidad de fármaco disuelto en el sitio de absorción llevándolo a concentraciones sobresaturadas y mejorando así su biodisponibilidad [15, 17, 19]. Las dispersiones sólidas se investigan mucho porque son muy versátiles en su aplicación y constituyen la base de preparados farmacéuticos aptos para ser administrados por diferentes vías y adecuados para diversas formas de dosificación, incluyendo la forma más popular, la tableta.

En la presente tesis se han tratado diversos aspectos asociados a la determinación experimental de algunas propiedades físico-químicas de las moléculas que son de interés en las etapas de *drug discovery*, la acidez, la solubilidad y la velocidad de disolución. El trabajo desarrollado se resume en cuatro capítulos que se describen muy someramente a continuación:

- **Capítulo 1:** La primera parte de este trabajo, se dedicó a validar metodología analítica para determinar las constantes de disociación ácida, valores de pK_a, en diversas mezclas metanol/agua y en un amplio intervalo de temperaturas, así como la variación de entalpía asociada a los procesos de desprotonación, ΔH, para algunas

sustancias representativas. Este trabajo se ha realizado porque estas magnitudes se requieren a menudo en los laboratorios farmacéuticos por sí mismas o como un paso obligatorio para la determinación de las propiedades de los fármacos tales como la solubilidad y el perfil de solubilidad con el *pH*. La razón de la utilización de disolventes de metanol/agua para las mediciones experimentales es la baja solubilidad de la mayoría de los fármacos con mayor potencial, los cuales exigen disolventes más eficaces que el agua pura (mezclas de metanol y agua) para obtener los valores de pK_a. Hay que señalar aquí que se dispone de ecuaciones adecuadas para la extrapolación que permiten una estimación fiable de los valores de pK_a acuoso incluso para compuestos muy insolubles. La razón para realizar mediciones en una amplia gama de temperatura está relacionada no sólo con el interés en conocer el comportamiento ácido-base de los compuestos a temperatura fisiológica, 37°C, sino también para obtener valores de consistentes de la variación de entalpía asociada a procesos de desprotonación de los principios activos.

- Capítulo 2: En la segunda parte de esta Tesis, se ha estudiado la solubilidad de algunos compuestos bioactivos y también de varios principios activos representativos. Como se ha explicado anteriormente, la solubilidad constituye un parámetro clave en el desarrollo de fármacos, y una solubilidad baja es una de las principales causas de la escasa biodisponibilidad de un fármaco. Sin embargo, la fracción de compuestos poco solubles que entran en fase de desarrollo clínico ha aumentado mucho durante la última década, tendencia que se refleja en el número mayor de compuestos BCS de Clase II y Clase IV en comparación con los de Clase I y Clase III. En consecuencia, se han desarrollado técnicas de formulación diversas que se centran en la mejora de la solubilidad de los compuestos, algunas de las cuales consideran la formación de sales y de co-cristales, sin cambiar la estructura de la fracción activa del fármaco en cuanto a su interacción con la diana. Sin embargo, el punto de partida para todas estas técnicas todavía se define a partir de la estructura de la molécula activa [24]. Las medidas de solubilidad son a menudo tediosas y consumen mucho tiempo y, de hecho, la literatura no ofrece un conjunto de datos de solubilidad de alta calidad para medicamentos, ya que muchas veces se publican valores significativamente diferentes para un mismo compuesto. Aunque hay

muchos modelos computacionales para predecir la solubilidad acuosa de moléculas orgánicas, estos modelos suelen mostrar resultados poco concordantes e incertidumbres grandes. El método clásico para la determinación de la solubilidad experimental es el de equilibración, conocido como *Shake-Flask* (S-F). Sin embargo, el método potenciométrico descrito por Avdeef es una buena alternativa para las mediciones de la solubilidad de la forma neutra de compuestos con propiedades ácido-base. Recientemente, Sirius Analytical Ltd. desarrolló un procedimiento basado en el método potenciométrico de Avdeef para medir la solubilidad de las moléculas ionizables de una forma más sencilla. En la presente Tesis se ha realizado un estudio amplio sobre las mejores condiciones experimentales para medir solubilidades mediante este método de acuerdo con el tipo de compuesto de interés.

- Capítulo 3: La tercera parte de este trabajo se refiere a la determinación del perfil de solubilidad de los fármacos con la acidez. Es bien conocido que el *pH* fisiológico de mayor interés es 7,4. Sin embargo, en el sistema gastrointestinal el *pH* varía desde 1-2 hasta 8, es decir, el *pH* del estómago varía aproximadamente desde 1,5 hasta 3,5, mientras que el del duodeno lo hace entre 5 y 7 y esta gran variación de *pH* condiciona mucho la solubilidad de compuestos ácidos o básicos. Por ejemplo, los compuestos débilmente básicos con valores de pK_a que van de 5 a 8 pueden ser completamente o parcialmente solubilizados en el estómago donde predominarán las especies ionizadas, pero pueden precipitar en las regiones bajas del tracto gastrointestinal, donde el *pH* aumenta significativamente y se forma la especie neutra de la base, de solubilidad mucho menor, tal como se ilustra esquemáticamente en la Fig. 1 [30]. Por esta razón, se han determinado los perfiles de solubilidad en función del *pH* de cinco fármacos ionizables de naturaleza química y terapéutica diferente. Se han utilizado dos métodos distintos: el método clásico de equilibración, S-F, y el método potenciométrico propuesto por Sirius Analytical Ld. con el fin de comprobar la consistencia de los resultados potenciométricos con los obtenidos por el método de referencia, S-F. La robustez del método potenciométrico, así como sus limitaciones, se han evaluado críticamente.

- Capítulo 4: La última parte de esta Tesis se ha desarrollado en Departamento de Tecnología Farmacéutica y Biofarmacia de la Universidad de Groningen (Holanda) bajo la supervisión del Prof. Hinrichs y se ha dedicado al estudio sobre la manera de mejorar la biodisponibilidad de Amphotericina B, un antibiótico y antifúngico potente pero muy insoluble, mediante el aumento de su velocidad de disolución. Este principio activo se ha tomado como fármaco modelo y se ha estudiado la velocidad de disolución de los comprimidos preparados a partir de dispersiones sólidas diversas del fármaco. Estas se han preparado utilizando el dimetil sulfóxido (DMSO) como disolvente y en presencia y en ausencia de fármaco-portador y también en presencia y ausencia de agentes tensioactivos. En cada caso se midió la velocidad de disolución de los comprimidos y se realizó un estudio comparativo. Esta parte del trabajo de Tesis constituye una parte de un estudio más amplio acerca de las diferentes estrategias para mejorar la biodisponibilidad de la Amphotericina B. Este trabajo se halla actualmente en curso en el laboratorio del Departamento de Tecnología Farmacéutica y Biofarmacia de la Universidad de Groningen y la parte descrita en esta tesis corresponde solamente a mi propia contribución al mismo.

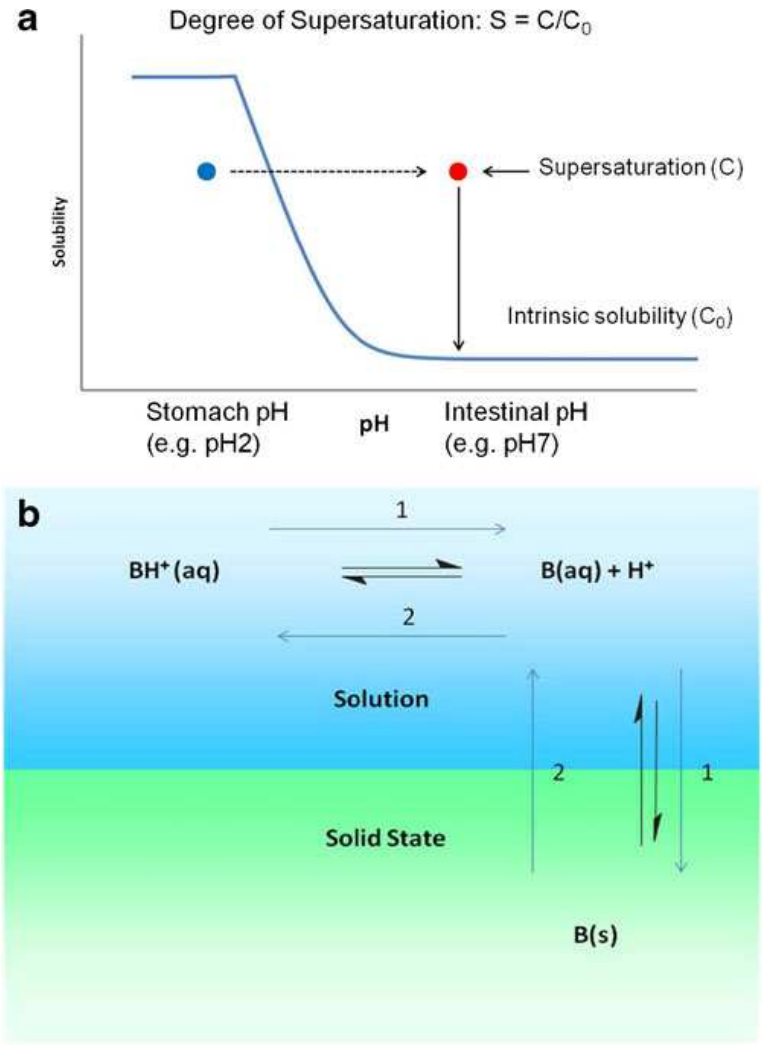

Figura. 1: Esquema de precipitación de un compuesto débilmente básico en el tracto gastrointestinal, GI: (a) Inicialmente, el compuesto está completamente solubilizado a un *pH* bajo. En las regiones de *pH* más alto del GI, la formación de la especie menos soluble, la base neutra, aumenta y por lo tanto disminuye la solubilidad. En consecuencia, el fármaco inicialmente completamente solubilizado en el estómago queda sobresaturado al entrar en la región intestinal. (b) Esquema de precipitación de fármaco que muestra la liberación o el consumo de protones en solución. Dirección 1: la precipitación base libre desplaza el equilibrio acuoso para sustituir a la especie B que desaparece de la solución. Por consiguiente se liberan protones en la solución y el *pH* disminuye. Dirección 2: la disolución de la base libre desplaza el equilibrio acuoso para producir más BH^+ y mantener la concentración de B (aq) cerca de la concentración de solubilidad. Por consiguiente se consumen protones de la solución y el *pH* aumenta.

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CHAPTER 1

Acidity constants of several neutral and cationic acids in aqueous and methanol/water solutions at various temperatures

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1.1 INTRODUCTION

Thermodynamic quantities such as equilibrium constants or enthalpy variations associated to the common chemical processes that occur in different conditions (i.e. solvent, temperature, ionic strength, etc.) are frequently required for the right interpretation of the chemical behavior of compounds. In particular, the reported increments of enthalpy (ΔH) for most acidic dissociation processes are referred to aqueous solutions and the temperature range of 15-40°C [1]. However, very often, literature shows discrepancies between the values obtained by different techniques and laboratories and it is not easy to select the best one for further calculation purposes. When specific values are not tabulated, it is mandatory to calculate them from the available data. The Van't Hoff equation is often used to estimate ΔH values of a particular process from the available acidity constants at different temperatures. This procedure involves the assumption that the ΔH quantity is constant in the temperature range considered.

It is much more difficult to have reliable data in hydroorganic solvent mixtures such as methanol/water, especially at any temperature different to 25°C. Nevertheless, methanol/water mixtures show very relevant applications such as suitable solvents for chemicals sparingly soluble in water or as mobile phases in liquid chromatography and, in both instances, it would be very useful to know the thermodynamic quantities involved in the acid-base reactions in these solvent mixtures. The first use has become very popular in drug discovery through the methodology proposed by Sirius Analytical, Ltd. to determine pK_a values of drugs by potentiometry [2, 3]. Thus, measurements of pK_a are done in a variety of methanol/water mixtures and the aqueous pK_a is easily obtained by extrapolation. The measurements and calculations are currently made at room temperature but the instruments and associated softwares are able to achieve pK_a values at higher temperatures such as 37 °C. However, the Sirius arrangements are unable to get accurate pK_a at temperatures higher than 40°C because of the limitations of the software, which is especially designed for the study of drugs. Nevertheless, this technology would be used to determine pK_a values at temperatures

higher than 40°C and different methanol/water mixtures if the suitable constants are used and a proper standardization procedure is performed.

Methanol/water mixtures have been also widely employed as mobile phases in liquid chromatography separations at room temperature separations. However, nowadays temperature has become a powerful analytical variable in HPLC and most of the modern chromatographs show devices to control the temperature along the separation process. All the published models to describe the effect of temperature on the retention for compounds with acid-base properties point out that retention depends on the acidic dissociation enthalpies and constants of both analyte and buffering agent [4-7]. These thermodynamic quantities should be referred to the binary solvent used as the mobile phase, methanol/water mixtures for instance, but they are rarely available in literature. The purpose of the present work is to establish an efficient tool to determine acidity constants in methanol/water binary solvents at different temperatures and, through the Van't Hoff equation, to be able to calculate the variation of enthalpy associated to the acidic dissociation process in these solvent mixtures.

To make matters more precisely, some comments about the terms and the methodology used in this chapter are defined subsequently.

1.1.1 Potentiometric determination of pK_a

To determine acidic dissociation constants potentiometrically by means of the Sirius methodology, the following items should be considered [2, 8].

1.1.1.1 *pH* scales

Operational *pH* scale is defined according to the familiar Nerst equation expressing by equation [1.1.1]

$$pH = pH_{(S)} - \frac{E - E_0}{2.303RT / F} \qquad [1.1.1]$$

where 2.303 RT/F is the Nerst slope, and *pH(s)* is the *pH* of standard buffer, ($E-E_0$) is the difference between potential of the sample and that of buffer solutions. For a given electrode, the *pH* value will slightly change over a period of weeks, but generally not appreciably in the time of a titration. Since the Sirius instrument, PCA101 analyzer, works in the concentration scale, it is necessary to convert the operational scale ($pH = -\log a_H$) to the concentration one ($p_cH = -\log\left[H^+\right]$). The relationship between two scales is:

$$pH = -\log a_H = p_cH - \log\gamma_H \qquad [1.1.2]$$

where γ_H is activity coefficient.

1.1.1.2 Standardization of the potentiometric system for pure water at 25°C

The four standardization parameters procedure has been used from the definition of *pH*, eq [1.1.2], and the effect of H^+ and OH^- ions on the liquid junction potential. The following equation was proposed [9].

$$pH = \alpha + Sp_cH + j_H\left[H^+\right] + j_{OH}\frac{K_W}{\left[H^+\right]} \qquad [1.1.3]$$

where α is expressed as:

$$\alpha = -\log\gamma_H + cte \qquad [1.1.4]$$

The α parameter is an adjustable parameter and mainly corresponds to the negative logarithm of the activity coefficient of H^+. *S*, is introduced to take into account that a

particular *pH* electrode may not have 100% Nernstian slope and its value should be nearly one in the ideal form. The j_H term corrects *pH* readings for the nonlinear *pH* response due to the liquid junction and asymmetry potentials in moderately acidic solution (*pH* = 1.5-2.5). The j_{OH} term corrects for high *pH* (*pH* >11) nonlinear effects, principally liquid junction in origin.

The autoionization constant of water, $K_W = \left[H^+\right]\left[OH^-\right]$ (in concentration scale), is incorporated into the software as a function of temperature and ionic strength. The α parameter and slope factor, S depend on the temperature, ionic strenght and, when any cosolvent is used, on the solvent mixture. After doing each blank standardization we have values of these four parameters.

Typical values of the adjustable parameters in the aqueous medium, at 25°C are:

$\alpha = (0.07 - 0.11) \pm 0.01$; $S = (0.999 - 1.005) \pm 0.001$; $j_H = 1.0 \pm 0.1$; $j_{OH} = -0.5 \pm 0.2$

1.1.1.3 Standardization of potentiometric system for mixtures of methanol/water at 25°C

For mixtures of methanol/water, standardization parameters values will change. So it is necessary to determine these new standardization parameters in each binary mixture. Sirius software embodies a multiterm equation for each standardization parameter that depends on the weight percentage and nature of the cosolvent. For the methanol/water mixtures and ionic strength of 0.15 M at 25°C, the following expressions,[1.1.5]–[1.1.8], derived from Sirius calculating program should be used.

$$\alpha = \alpha_o - 0.09983w + 2.1366w^2 - 2.1930w^3 \qquad [1.1.5]$$

$$S = S_o - 0.01153w - 0.12122w^2 + 0.17575w^3 \qquad [1.1.6]$$

$$j_H = j_{OH} \quad [1.1.7]$$

$$j_{OH} = j_{o\,OH} + 3.247w \quad [1.1.8]$$

where $_o$, S_o, j_{OH} and $j_{o\,OH}$ are obtained parameters from the blank standardization in the aqueous medium and w stands for the weight percentage of methanol. PCA101 analyser could obtain these standardization parameters by using *pK$_a$LOGP*^{ITM} software in any mixture of methanol/water at 25°C.

1.1.1.4 Determination of thermodynamic acidity constants of weak acids

An acidic dissociation constant is a particular example of an equilibrium constant. For the specific equilibrium between a monoprotic acid, HA, and its conjugate base, A^-, in pure water,

$$HA(aq) + H_2O \longrightarrow H_3O^+(aq) + A^-(aq)$$

The thermodynamic equilibrium constant, K_a can be defined by:

$$K_a = \frac{\left[H_3O^+\right]\left[A^-\right]}{\left[HA\right]} \times \frac{\gamma_{H_3O^+}}{\gamma_{HA}} = K_a^c \times \Gamma$$

and

$$pK_a = pK_a^c - \log\Gamma \quad [1.1.9]$$

being K_a^c the concentration constant and Γ the quotient of activity coefficients. PCA101 analyzer calculates pK_a^c values. The $\log\Gamma$ term is calculated from the extended Debye–Hückel expression relating single-ion activity coefficients to ionic strength that is:

$$\log\gamma_{\pm} = -Az^2 \frac{I^{1/2}}{1 + a_o B I^{1/2}} \qquad [1.1.10]$$

where, in the aqueous solution, the parameters A, B are 0.51 and 0.32 (molar scale) respectively at 25°C, a_o=4.56 Å, z is the charge of the ionic species and I is the ionic strength [8].

Acording to the IUPAC recommendations, values of the thermodynamic dissociation constants in organic or hydro organic solvents are symbolized by ${}^{s}_{s}pK_a$ and consequently, the symbol, ${}^{w}_{w}pK_a$, is used for dissolution constants in aqueous solutions. These symbols will be used in this work. Thus, lower-case left-hand superscripts indicate the solvent (w) to a nonaqueous or mixed solvents in which measurements are being made; lower-case left-hand subscripts indicate the solvent in which the ionic activity coefficient γ_i is reffered to unity at infinite dilution (w or s) [10].

To determine ${}^{s}_{s}pK_a$ values of acidic constants in methanol/water mixtures, the procedure is the one described for aqueous solution but suitable standardization parameters and also coefficients A and a_oB of the Debye-Hückel equation should be used.

Literature shows several extrapolation equations to estimate ${}^{w}_{w}pK_a$ from the ${}^{s}_{s}pK_a$ values determined in various methanol/water mixtures. The most popular is the Yasuda–Shedlovsky one that relates ${}^{s}_{s}pK_a$ to the reverse of the dielectric permittivity of the binary solvent , ε^{-1}, by means of Eq [1.1.11].

$$ {}^{s}_{s}pK_a + \log[H_2O] = a_\varepsilon \varepsilon^{-1}_{MeOH/H_2O} + b_\varepsilon \quad [1.1.11] $$

where a_ε and b_ε are empirical constants that depend on each substance and temperature [2,3,11-20]. Eq [1.1.11] requires ${}^{s}_{s}pK_a$ values at various methanol percentages to evaluate a_ε and b_ε and it allows the estimation of ${}^{w}_{w}pK_a$ using ε =78.3 and $[H_2O]$=55.5 that is, the constants values corresponding to pure water. It means ${}^{w}_{w}pK_a$ is ${}^{s}_{s}pK_a$ value when ε =78.3. The highest methanol content to keep the linearity is about 60% of methanol in weight [8, 11] since the Eq [1.1.11] is valid for solutions with $\varepsilon > 50$.

1.2 EXPERIMENTAL

1.2.1 Instrument

The PCA101 chemical analyzer, developed and manufactured by Sirius Analytical Instruments Ltd, is the first commercial instrument designed specifically to determine ionization constants, $pK_{a,}$ and oil water partition coefficients, log *P,* of weak acids and bases [21, 22]. A heated water bath with circulator will be required for constant temperature work.

The PCA101 should be used with argon to protect samples absorbing atmospheric carbon dioxide. The argon is delivered to the sample vial using the argon cap. If argon is not available, nitrogen or helium can be used.

It is supplied with a Sirius, Ag/AgCl double junction, fixed lead electrode (PCA0028). The PCA 101 controls the syringe, the 2-way valve, the 6-way valve which one is connected to the one reagent and the movements of the dispenser tip which is made from a quartz capillary with very small internal diameter to minimise diffusion of reagent or sample while the tip is immersed in the solution. The system is shown in Figs. 1.2.1 and 1.2.2.

Figure 1.2.1: PCA 101

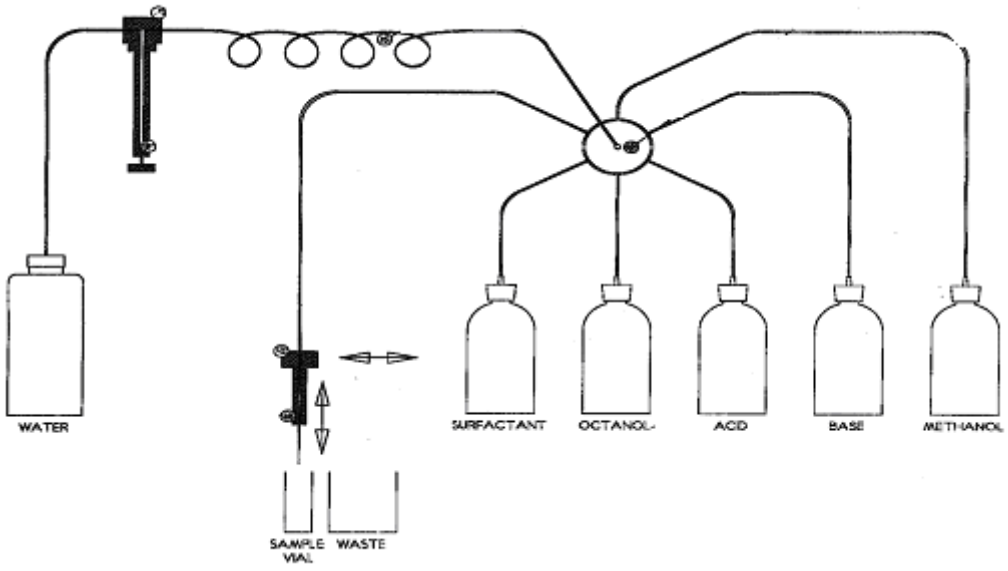

Figure 1.2.2: Diagrammatic layout of PCA101 fluidics system

The processing of the *pH*-metric data, computations of ${}^{s}_{s}pK_a$ values via a nonlinear least squares procedure and Yasuda-Shedlovsky extrapolation treatments, were performed using pK_aLOGP$^{\Pi M}$ software (V 5.02, 2001 Sirius). This methodology is prepared to work at ionic strength of 0.15 M KCl.

1.2.2 Reagents and Solvents

•Buffer solution *pH* 7.00 (Potassium dihydrogen phosphate, KH_2PO_4, disodium Hidrogen phosphate $Na_2HPO_4.12H_2O$), Crison

• Hydrochloric acid 0.5 M, Merck, Titrisol®;

• Potassium hydroxide 0.5 M, Merck, Titrisol®;

• Potassium chloride, Merck, > 99.5%

• Potassium biphtalate, Merck, > 99.8%

• Methanol, Merck, HPLC grade

• Water purified by a Milli-Q plus system from Millipore with resistance higher than 18 MΩ.

1.2.3 Studied substances

- Aniline, Merck, > 99.5%
- Tris (hydroxymethyl) aminomethane, Aldrich, > 99.99%
- Sodium Benzoate, Panreac, > 96%
- Sodium Acetate, Carlo Ebra, > 99%

1.2.4 Procedure

1.2.4.1 Electrode Calibration

The objective of this step is to convert emf readings which are in millivolts to *pH* values. At the start of each assay, the PCA 101 takes a millivolt reading from PCA0028 electrode, E_s, of a buffer solution *pH* 7.00. This buffer is chosen to coincide with iso-potential point of the *pH* electrode; consequently, E_s is ideally temperature independent. From this reading the operational *pH* scale is defined according to the Nerst equation [1.1.1].

1.2.4.2 Four standardization parameters of potentiometric system

The simplest standardization procedure is to titrate with standardized 0.5 M KOH a 0.15 M KCl acidified with a precisely known amount of HCl (enough to lower *pH* to about 1.8, which is 0.8-0.9 mL 0.5 M per 20mL 0.15 M KCl) up to about *pH* 12.2 (which consumes about 2mL 0.5 M KOH). This is called blank titration**.**

Standardization system was performed in aqueous solution and also in several methanol/water mixtures. All titrations in aqueous solutions were made at different temperature range from 25-55°C.

1.2.4.3 Determination of ${}^{s}_{s}pK_a$ values in several mixtures of methanol/water (0−60% w/w) at different temperatures (25−55)°C

All titrations were performed in solutions of 0.15 M KCl under nitrogen atmosphere at different temperatures from (25 ± 0.5) to (55 ± 0.5)°C. Everyday after doing electrode standardization, blank and set up four standardization parameters we start to do main titration measurements. A series of samples of 0.01 g dissolved in the 15 mL total of hidroorganic solution (mixtures of methanol/water with different percentage, 0−60% w/w of methanol) were preacidified to *pH* 1.8 with 0.5 M aqueous HCl and titrated with 0.5 M aqueous KOH to *pH* 12.2. Each sample was titrated at six different percentages of methanol. The overall procedure was carried out at least two times for each compound.

1.2.4.4 Calculations

Potentiometric ${}^{s}_{s}pK_a$ values (I=0.15) of anilinium, protonated tris ($HTris^{+}$) , benzoic and acetic acids at various methanol/water compositions and temperatures were calculated from the potentiometric curves by means of the pK_aLOGP^{ПМ} software (V5.2 Sirius). The Yasuda-Shedlovsky equation allowed the interpolation of ${}^{s}_{s}pK^{c}_{a}$ at methanol/water mixtures of 10, 20, 30, 40, 50 and 60% (w/w) of methanol. Obtained values were converted into the thermodynamic ones (I=0) by means of Debye-Hückel equation [1.1.10].

1.3 RESULTS & DISCUSSION

The selected compounds have been two neutral acids, acetic and benzoic, and two neutral bases, aniline and tris (hydroxymethyl) aminomethane. It is always necessary to do standardization of the system before determining potentiometrically any acidity constant.

1.3.1 System standardization in several mixtures of methanol/water at 25°C

The four standardization parameters in aqueous solution at 25°C and ionic strength of 0.15 M KCl have been determined. As it is known, in mixtures of methanol/water, the four standardization parameters depend on the percentage of methanol. So these four parameters have been determined by means of Eqs [1.1.5]–[1.1.8]. Moreover, in order to evaluate the accuracy of these equations each one of these four parameters has been determined directly by blank titrations in several methanol/water mixtures. Fig. 1.3.1 shows the obtained results.

As you can see in Fig. 1.3.1, there is concordance between experimental and calculated values derived by equations [1.1.5]−[1.1.8] for parameters α, S and j_H. However, experimental parameters present a slight discrepancy with those calculated when the percentage of methanol increases. The discrepancy in j_{OH} parameter is higher and increases with the methanol content too. Because of the j_{OH} is significant only in very basic solutions (pH >11) this parameter affects very little the calculated pK_a values and this discrepancy has a little effect on results. So, we can accept calculated values as reliable standardization parameters in different weight percentage of methanol (0-60%) at 25°C .

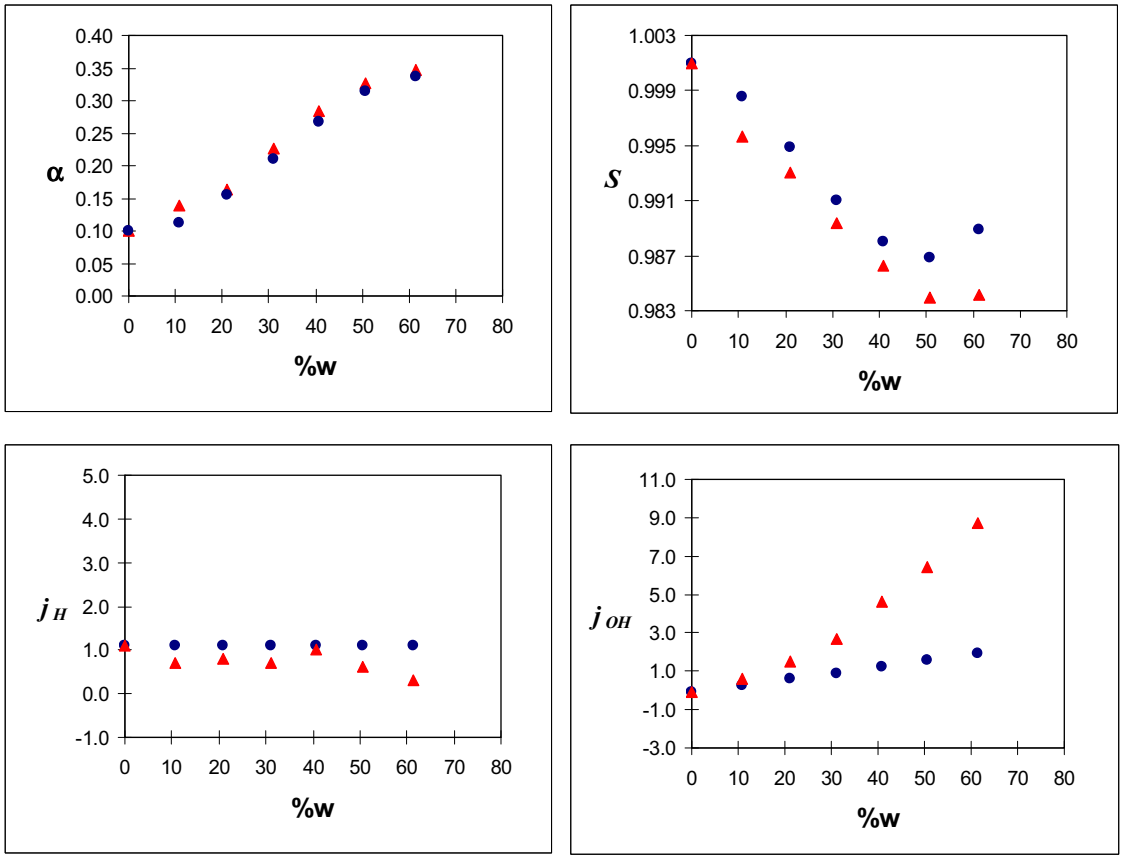

Figure 1.3.1 Parameters of standardization vs. weight percentage of methanol (I=0.15 in KCl at 25°C) ▲ are experimental values of standardization parameters in different blanks of methanol/water mixtures at 25°C ● are calculated values derived by equations [1.1.5]-[1.1.8]

1.3.2 System standardization in several mixtures of methanol/water at different temperatures (25-55)°C

As we are interested to work at higher temperatures, it is necessary to carry out the experimental titrations using the suitable values for the standardization parameters α, S, j_H and j_{OH}. These values have been determined in this work from 25 to 55°C in aqueous solution. Each determination was done by triplicate and the mean values reported in Fig. 1.3.2 and in Table 1.3.1.

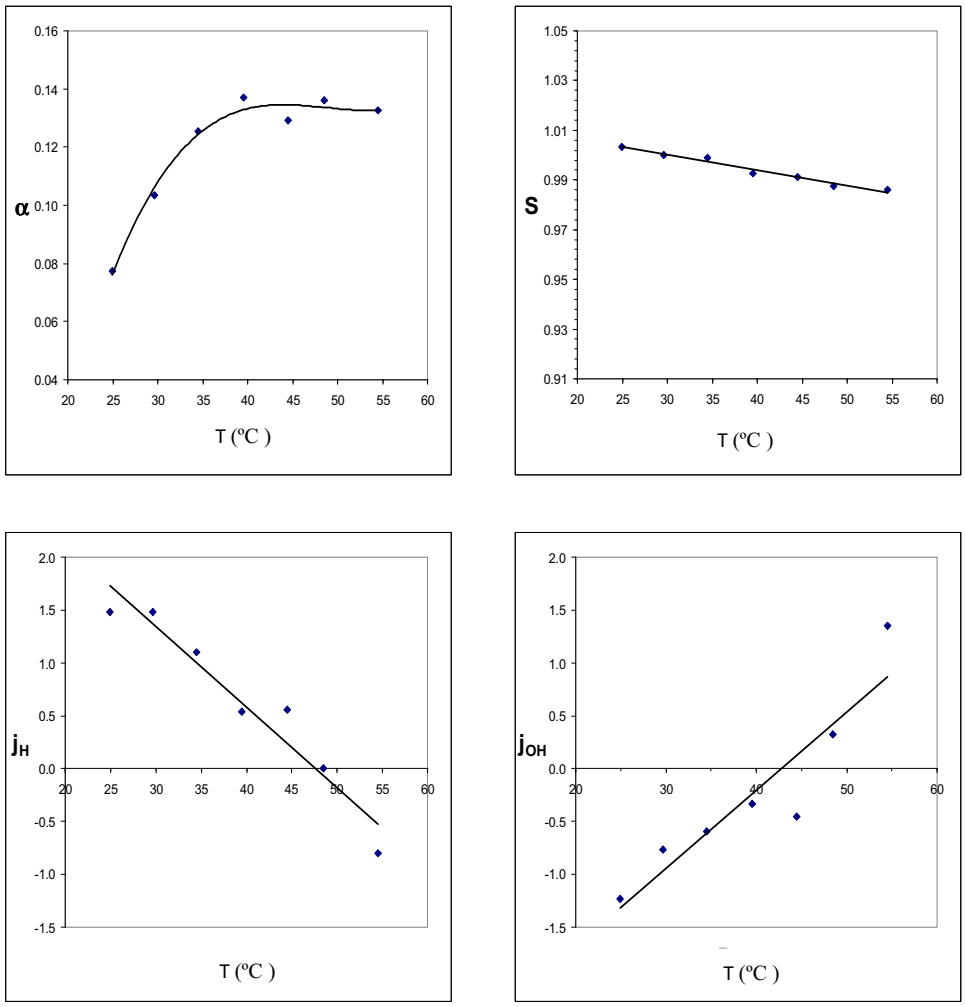

Figure 1.3.2 Temperature dependence of the four standardization parameters in aqueous solution

Table 1.3.1 Mean values of four standardization parameters at different temperatures (25-55°C) in aqueous solution

T (°C)	*α*	*s*	j_H	j_{OH}
24.9	0.08 ± 0.02	1.003 ± 0.001	1.5 ± 0.3	-1.2 ± 0.4
29.6	0.10 ± 0.01	1.000 ± 0.002	1.5 ± 0.4	-0.8 ± 0.4
34.5	0.13 ± 0.03	0.999 ± 0.003	1.1 ± 0.5	-0.6 ± 0.3
39.5	0.14 ± 0.02	0.993 ± 0.002	0.5 ± 0.4	-0.3 ± 0.3
44.5	0.13 ± 0.05	0.991 ± 0.002	0.6 ± 0.4	-0.5 ± 0.2
48.5	0.14 ± 0.06	0.988 ± 0.001	0.0 ± 0.4	0.3 ± 0.3
54.5	0.13 ± 0.08	0.986 ± 0.001	-0.8 ± 1.5	1.4 ± 0.3

The α values increase as temperature increases until 40°C but from 40°C to 55°C there isn't any dependency on temperature and α value remains constant. The *S* value sligthly increases as temperature decreases and j_H shows the same trend but its line has a sharper slope. It means this last parameter changes greatly with the variation of temperature. j_{OH} parameter increases as temperature increases and the significance of this change is similar to that of the j_H .

At this point, since α and *S* parameters, (the main parameters in Eq. [1.1.13]) change slightly with temperature, we make the hypothesis that we can use Eqs. [1.1.5]-[1.1.8] to estimate the standardization parameters in methanol/water mixtures at any temperature (25-55)°C range. Therefore, values of parameters determined in aqueous solution at different temperatures and Eqs. [1.1.5]-[1.1.8] have been used in the standardization of the potentiometric systems.

1.3.3 Determination of ${}^{s}_{s}pK_a$ values of studied compounds in the mixtures of methanol/water (0−60% w/w) at different temperatures (25−55)°C

The dissociation constants of the selected compounds have been potentiometrically determined in the 0-60% methanol percentage at different temperature from (25-55)°C range. Obtained values are shown in Tables 1.3.2 and 1.3.3.

Table 1.3.2 Experimental aqueous pK_a values at I = 0.15 M for benzoic acid, acetic acid, $HTris^+$ and anilinium at different temperatures (25-55°C)

Benzoic acid		Acetic acid		$TrisH^+$		Anilinium	
T (°C)	pK_a (I)	T (°C)	pK_a (I)	T (°C)	pK_a (I)	T (°C)	pK_a (I)
25.1	3.99	25.2	4.582	24.9	8.163	25.0	4.69
29.6	3.98	30.4	4.543	29.9	8.016	29.1	4.58
34.4	4.01	34.9	4.555	34.8	7.915	34.8	4.52
39.9	4.06	39.8	4.572	39.8	7.839	39.8	-
44.5	4.08	44.5	4.621	44.5	7.790	44.5	4.44
49.5	4.11	49.5	4.696	48.5	7.769	48.5	4.39
54.5	4.05	54.5	4.794	54.5	7.653	54.5	4.29

Table 1.3.3 Experimental ${}^{s}_{s}pK_a$ values at I = 0.15 M for benzoic acid, acetic acid, $HTris^+$ and anilinium at several mixtures of methanol/water and different temperatures (25-55°C)

Benzoic acid			Acetic acid			$HTris^+$			Anilinium		
T (°C)	MeOH% (w/w)	pK_a	T (°C)	MeOH% (w/w)	pK_a	T (°C)	MeOH% (w/w)	pK_a	T (°C)	MeOH% (w/w)	pK_a
25.1	8.68	4.08	25.2	10.04	4.66	24.8	10.03	8.16	25.1	9.94	4.63
25.2	17.76	4.28	25.2	19.78	4.83	25	19.93	8.13	25.4	19.71	4.59
25.3	25.8	4.47	25.2	29.26	4.99	25.3	29.25	8.11	25.6	28.93	4.52
25.3	34.98	4.69	25.1	38.16	5.19	25.7	38.41	8.09	25.5	37.78	4.44
25.4	42.23	4.85	25.3	38.52	5.20	25.7	47.43	8.05	25.4	46.91	4.36
25.2	51.64	5.11	25.2	47.36	5.37	25.6	56.77	8.01	25.3	55.89	4.26
29.6	10.00	4.14	30.4	9.86	4.72	29.9	9.85	8.02	28.6	9.93	4.57
29.5	19.85	4.34	30.5	19.66	4.83	30	9.98	8.01	28.9	19.77	4.48
29.5	29.3	4.57	30.5	28.9	4.984	30	19.96	7.94	29	28.65	4.44
29.5	38.85	4.76	30.5	37.2	5.17	30	29.48	7.92	29.1	38.05	4.34
29.5	49.41	5.03	30.5	48.46	5.37	30.4	38.56	7.93	30.1	47.43	4.23
29.8	55.94	5.20	30.5	57.99	5.50	30.3	38.67	7.93	30.2	54.06	4.19
						30.2	38.85	7.91			
						29.5	39.01	7.92			
						30.4	49.11	7.91			
						29.9	49.53	7.84			
						29.2	49.94	7.90			
						30.3	58.64	7.89			
34.3	8.69	4.12	34.8	10.1	4.68	34.9	9.83	7.91	35	9.89	4.44
34.5	10.00	4.16	34.6	10.24	4.69	34.9	19.38	7.90	35	19.68	4.40
34.4	17.15	4.30	34.9	19.84	4.80	34.9	28.83	7.84	34.8	28.72	4.31
34.5	28.41	4.54	34.7	20.45	4.87	34.9	38.42	7.79	34.9	37.57	4.26
34.5	29.1	4.61	34.7	30.34	4.97	34.9	40.89	7.78	34.8	48.72	4.15
34.3	33.73	4.69	34.7	41.04	5.16	35	47.52	7.71	34.9	58.07	4.06
34.3	38.49	4.78	34.7	51.12	5.33	35	49.39	7.73			
34.5	47.26	5.02	34.6	59.53	5.51	35	55.86	7.73			
34.5	50.95	5.07				35.1	58.51	7.73			
34.6	56.20	5.23									
34.5	61.75	5.28									
39.9	9.61	4.25	39.8	9.47	4.70	39.8	9.8	7.83	39.6	9.32	4.38
39.9	18.83	4.43	39.9	9.55	4.76	39.8	19.58	7.76	39.6	18.71	4.32
39.9	28.82	4.62	39.8	18.97	4.87	39.8	28.29	7.79	39.6	28	4.23
39.9	39.73	4.87	39.9	28.40	5.00	39.8	37.42	7.74	39.7	39.5	4.16
39.9	48.78	5.06	39.9	35.89	5.18	39.8	48.09	7.71	39.7	46.95	4.11
39.9	57.43	5.26	39.7	38.05	5.23	39.8	57.41	7.66	39.7	55.79	4.05
			39.9	39.68	5.23						
			39.7	47.51	5.35						
			39.6	56.33	5.53						

Table 1.3.3 Cont. Experimental ${}^{s}_{s}pK_a$ values at I = 0.15 M for benzoic acid, acetic acid, $HTris^+$ and anilinium at several mixtures of methanol/water and different temperatures (25-55)°C

Benzoic acid			Acetic acid			$HTris^+$			Anilinium		
T (°C)	MeOH% (w/w)	pK_a	T (°C)	MeOH% (w/w)	pK_a	T (°C)	MeOH% (w/w)	pK_a	T (°C)	MeOH% (w/w)	pK_a
44.5	8.53	4.18	44.5	10.22	4.77	44.5	10.07	7.74	44.5	9.76	4.34
44.5	17.09	4.36	44.5	20.23	4.98	44.5	19.63	7.70	44.5	19.63	4.32
44.5	22.8	4.42	44.5	29.99	5.15	44.5	19.64	7.64	44.5	29.25	4.20
44.5	25.38	4.59	44.5	39.14	5.33	44.5	29.02	7.77	44.5	40.49	4.12
44.5	32.88	4.78	44.5	47.84	5.50	44.5	29.33	7.73	44.5	49.21	4.05
44.5	34.43	4.73	44.5	54.49	5.44	44.5	39.1	7.68	44.5	59.25	3.95
44.5	34.98	4.66	44.5	56.95	5.55	44.5	40.6	7.70			
44.5	48.38	4.97	44.5	57.95	5.68	44.5	49.18	7.68			
44.5	56.23	5.24	44.5	58.33	5.50	44.5	49.95	7.65			
44.5	63.62	5.39	44.5	58.73	5.54	44.5	50.06	7.66			
			44.5	58.77	5.54	44.5	56.9	7.52			
						44.5	59.7	7.48			
48.5	10.04	4.31	49.5	9.82	4.86	49.5	10.04	7.78	49.5	9.99	4.34
49.5	19.87	4.54	49.5	19.88	4.97	49.5	19.85	7.65	49.5	19.86	4.29
49.5	20.26	4.44	49.5	29.27	5.15	49.5	19.87	7.67	49.5	29.64	4.18
49.5	30.85	4.66	49.5	38.76	5.30	49.5	19.95	7.65	49.5	38.84	4.11
49.5	38.92	4.84	49.5	49.4	5.45	49.5	20.14	7.73	49.5	48.01	4.04
49.5	39.08	4.93	49.5	59.98	5.68	49.5	29.6	7.62	49.5	60.01	3.95
49.5	48.05	5.13				49.5	29.92	7.69			
49.5	57.38	5.33				49.5	39.49	7.73			
						49.5	39.63	7.65			
						49.5	50.82	7.66			
						49.5	60.62	7.64			
54.5	10.17	4.23	54.5	9.9	4.80	54.5	10.04	7.64	54.5	10.01	4.22
54.5	20.13	4.44	54.5	9.96	4.82	54.5	20.14	7.63	54.5	19.33	4.16
54.5	29.96	4.63	54.5	20.12	5.00	54.5	29.96	7.56	54.5	29.47	4.20
54.5	34.89	4.79	54.5	29.58	5.08	54.5	39.69	7.56	54.5	39.15	4.07
54.5	39.64	4.82	54.5	29.68	5.25	54.5	50.99	7.50	54.5	50.16	4.03
54.5	44.56	4.90	54.5	39.42	5.34	54.5	61.66	7.43	54.5	58.31	3.89
54.5	51.40	5.06	54.5	49.18	5.54						
54.5	53.47	5.10	54.5	61.31	5.72						
54.5	61.70	5.30									

In order to get interpolated values at more common methanol percentages (0, 10, 20, 30, 40, 50%), the Yasyda-Shedlovsky equation (Eq [1.1.11]) has been used . In order to be able to use Yasuda-Shedlovsky equation, the dielectric constant (ε) at the mentioned methanol percentage and temperature must be included. Morever, the density (d) values of the pure methanol and pure water at each temperature are needed to calculate the water concentration value in each methanol/water mixture.

1.3.3.1 Variation of dielectric constants and densities with solvent composition and temperature

It is important to know that PCA101 software is prepared to work only between temperatures 25–40°C. So we need an enlarged equation for calculating dielectric constants (ε) in order to achieve good results at higher temperatures using Yasuda-Shedlovsky equation (Eq. [1.1.11]). Therefore, values of dielectric constants at 25-60°C temperature range by using Eq.[1.3.1] was derived from literature [23].

$$\varepsilon_{MeOH/H_2O(T)} = 10^{\delta} + \log D_0 \qquad [1.3.1]$$

where

$$D_o = 78.47 - 0.42w\% - 5.1\times10^{-4}(w\%)^2 \qquad [1.3.2]$$

and

$$\delta = (t - 25)\left[-0.020 - 0.0005w\% / 60\right] \qquad [1.3.3]$$

where w% is the percent weight fraction of methanol and t is temperature in Celsius scale. In the same manner, values of densities of pure water and pure methanol in the temperature range from (0−50)°C were originated from the following expressions [24]:

$$d_{MeOH} = -6.2185\times10^{-7}t^2 - 9.2062\times10^{-4}t + 8.1022\times10^{-1} \qquad [1.3.4]$$

$$d_{H_2O} = -4.9356\times10^{-6}t^2 + 7.6359t + 9.9998\times10^{-1} \qquad [1.3.5]$$

where *d* stands for the density of the subscript species at t temperature in Celsius scale. Suitable interpolated values of $d_{H_2O(T)}$, $d_{MeOH(T)}$ and $\varepsilon_{MeOH/H_2O(T)}$ have been used in the application of the Yasuda−Shedlovsky (Eq [1.1.11]) which relates the pK_a of an acidic compound in any methanol/water mixture ($^{s}_{s}pK_a$) to the dielectric permittivity of the solvent.

1.3.3.2 Effect of temperature on Debye–Hückel parameters

In order to be able to calculate the thermodynamic pK_a values, the Debye-Hückel approach has been used. Parameters of Debye–Hückel, Eq [1.1.10], change if temperature value changes and the product a_oB can be estimated by the following equation [25-28]:

$$(a_oB)_T = 1.5\left[\left(\varepsilon_w d_s\right)/\left(\varepsilon_s d_w\right)\right]_T^{0.5} \qquad [1.3.6]$$

where ε_w, d_w show the dielectric constant and the density of water, respectively, and ε_s and d_s show the dielectric constant and density of any methanol/water mixture. All these values are reffered to the given temperature. d_s values were interpolated from experimental data given in [24].

The A parameter has been computed from the following equation [29].

$$A_T = 1.8246 \times 10^6 / \left(\varepsilon T\right)^{3/2} \qquad [1.3.7]$$

In Table 1.3.4 we have the Debye-Hückel parameters calculated by using Eqs [1.3.6] and [1.3.7].

Table 1.3.4 The Debye–Hükel parameters at different temperatures (15–55°C) methanol percentage is given in weight

	0% MeOH		10% MeOH		20% MeOH		30% MeOH		40% MeOH		50% MeOH	
T (°C)	A	a_0B	A	a_0B	A	a_0B	A	a_0B	A	a_0B	A	a_0B
15.0	0.50	1.50	0.54	1.54	0.59	1.59	0.65	1.64	0.72	1.69	0.81	1.76
25.0	0.51	1.50	0.55	1.54	0.60	1.59	0.67	1.64	0.74	1.70	0.84	1.77
30.0	0.51	1.50	0.56	1.54	0.61	1.59	0.68	1.64	0.76	1.70	0.85	1.77
35.0	0.52	1.50	0.57	1.54	0.62	1.59	0.69	1.65	0.77	1.70	0.87	1.78
40.0	0.53	1.50	0.57	1.54	0.63	1.59	0.69	1.65	0.78	1.71	0.88	1.78
45.0	0.53	1.50	0.58	1.54	0.64	1.59	0.71	1.65	0.79	1.71	0.89	1.79
50.0	0.54	1.50	0.59	1.55	0.65	1.59	0.72	1.65	0.81	1.72	0.91	1.79
55.0	0.55	1.50	0.59	1.55	0.66	1.59	0.73	1.66	0.82	1.72	0.93	1.79

1.3.3.3 Dissociation constants

Yasuda-Shedlovshy equation (Eq. [1.1.11]) has been used to interpolate the ${}^{s}_{s}pK_a$ values at 10, 20 30, 40 and 50% of methanol and then, they have been converted into the thermodynamic ones by means of the Debye-Hückel approach (Eq. 1.1.10).

Tables (1-1A) and (1-2A) in Appendix show the conversion of pK_a (I) values to thermodynamic pK_a ones for the interpolated values. Note that for anilinium and $HTris^+$ no conversion is necessary.

Table 1.3.5 Thermodynamic acidic dissociation constants at different temperatures at ionic strength I=0 for bases

		Values in water			Values in MeOH/water 50% (w/w)		
	T (°C)	pK_a (Pot.)	pK_a (ITC)	pK_a (Lit.)[a]	pK_a (Pot.)	pK_a (ITC)	pK_a (Lit.)
Anilinium	15.0		4.79 ± 0.01	4.78		4.46 ± 0.03	
	20.0			4.7			
	25.0	4.69 ± 0.01	4.62 ± 0.01	4.62		4.32 ± 0.01	
	25.4				4.33 ± 0.01		
	29.1	4.58 ± 0.01					
	29.3				4.23 ± 0.02		
	30.0			4.51			
	34.8	4.52 ± 0.01					
	34.9				4.15 ± 0.01		
	35.0		4.46 ± 0.01	4.43		4.13 ± 0.04	
	39.8						
	40.0			4.35			
	44.5	4.44 ± 0.01			4.04 ± 0.02		
	45.0		4.35 ± 0.02	4.27		4.03 ± 0.02	
	48.5	4.39 ± 0.01					
	49.5				4.03 ± 0.03		
	50.0						
	54.5	4.29 ± 0.01			3.99 ± 0.03		
	55.0		4.17± 0.02			4.00 ± 0.04	
	65.0		4.09 ± 0.01				
HTris$^+$	15.0			8.36			
	24.9	8.16 ± 0.01					
	25.0			8.07			7.82
	25.4				8.04 ± 0.01		
	29.9	8.02 ± 0.01					
	30.4				7.90 ± 0.01		
	34.8	7.92 ± 0.01					
	35.0			7.80	7.75 ± 0.02		
	39.8	7.84 ± 0.01			7.70 ± 0.02		
	44.5	7.79 ± 0.01			7.61 ± 0.05		
	45.0			7.55			
	48.5	7.77 ± 0.01					
	54.5	7.65 ± 0.01			7.50 ± 0.02		
	55.0			7.32			

Table 1.3.5 Cont. Thermodynamic acidic dissociation constants at different temperatures at ionic strength of I=0 for acids

		Values in water			Values in MeOH/water 50% (w/w)		
	T (°C)	pK_a (Pot.)	pK_a (ITC)	pK_a (Lit.)[a]	pK_a (Pot.)	pK_a (ITC)	pK_a (Lit.)
Benzoic Acid	15.0		4.17 ± 0.01	4.21		5.33 ± 0.03	
	20.0			4.21			5.43[b]
							5.43[b]
	25.0		4.23 ± 0.03	4.2		5.38 ± 0.06	5.39[a]
	25.1	4.24 ± 0.01					
	25.2				5.47 ± 0.03		
	29.6	4.24 ± 0.01			5.45 ± 0.03		
	30.0						5.42[b]
	34.4	4.27 ± 0.01			5.49 ± 0.03		
	35.0		4.28 ± 0.02	4.21		5.39 ± 0.01	5.41[b]
	39.9	4.33 ± 0.01			5.42 ± 0.03		
	40.0						5.42[b]
	44.5	4.37 ± 0.01			5.46 ± 0.03		
	45.0		4.28 ± 0.03			5.32 ± 0.25	5.42[b]
	49.5	4.38 ± 0.01			5.53 ± 0.02		
	50.0			4.22			5.42[b]
	54.5	4.33 ± 0.01			5.46 ± 0.02		
	55.0		4.33 ± 0.03			5.34 ± 0.01	
	60.0			4.24			
	65.0		4.27 ± 0.02				
Acetic Acid	15.0			4.76		5.55 ± 0.04	
	20.0			4.76			
	25.0			4.76		5.61 ± 0.04	5.66[a]
	25.2	4.84 ± 0.01			5.84 ± 0.03		
	30.0			4.76			
	30.4	4.81 ± 0.01					
	30.5				5.83 ± 0.02		
	34.7				5.76 ± 0.02		
	34.9	4.82 ± 0.01					
	35.0			*4.76*			
	39.8	4.84 ± 0.01			5.83 ± 0.02		
	40.0			4.77			
	44.5	4.89 ± 0.01			5.90 ± 0.07		
	45.0			4.78		5.65 ± 0.09	
	49.5	4.97 ± 0.01			5.92 ± 0.03		
	50.0			4.78			
	54.5	5.07 ± 0.01			5.95 ± 0.05		
	55.0			4.80		5.79 ± 0.07	
	60.0			4.81			

[a] from reference1; [b] from reference 28

To validate the obtained results, ${}^{s}_{s}pK_a$ values have been compared with those from literature at 0 and 50% of methanol/water mixtures (Table 1.3.5). In each instances, literature values are classified into two different columns. The first one is devoted to ${}^{s}_{s}pK_a$ values obtained by means of isothermal titrations in our laboratory. The second column shows values obtained by different methods and published by a variety of authors.

Experimental and literature results show an excellent agreement and this fact confirms the hypothesis that Eqs [1.1.5]-[1.1.8] can be successfully applied to calculate the four standardization parameters at 25-55°C temperature range in methanol/water mixtures until 60% in weight percentage of methanol. This consistency validates the potentiometric procedure at temperatures higher than 25°C and, consequently, validates also the whole set of the potentiometric pK_a values shown in Table 1.3.5.

${}^{s}_{s}pK_a$ values at different methanol content solutions and temperatures calculated from the experimental data by means of the Yasuda- Shedlovsky equation (Eq 1.1.11) are reported in Table 1.3.6 which is a summary of values given in Table (1-1A)-(1-2A) in appendix. The graphical presentation of these data is shown in Figs. 1.3.3 and 1.3.4 for obtained values at 25°C and 50°C, respectively.

The studied compounds are acids and bases. As expected ${}^{s}_{s}pK_a$ values for acids increase by increasing the percentage of methanol. In contrast to the bases ${}^{s}_{s}pK_a$ values decrease by increasing the percentage of methanol [30].

In the study of how ${}^{s}_{s}pK_a$ change values with temperature, we can see that ${}^{s}_{s}pK_a$ values of benzoic and acetic acids don't change extremely as temperature increases and we can consider them as almost constants values. However ${}^{s}_{s}pK_a$ values for protonated amines like tris and aniline decrease considerablely as temperature increases.

Table 1.3.6 Potentiometric ${}^{s}_{s}pK_a$ interpolated from the experimental values at various methanol/water compositions (methanol percentage is given in weight).

	T (°C)	10%	20%	30%	40%	50%
Anilinium	25.4	4.63 ± 0.01	4.58 ± 0.01	4.51 ± 0.01	4.43 ± 0.01	4.33 ± 0.01
	29.3	4.55 ± 0.02	4.49 ± 0.02	4.42 ± 0.02	4.34 ± 0.02	4.23 ± 0.02
	34.9	4.44 ± 0.01	4.39 ± 0.01	4.32 ± 0.01	4.24 ± 0.01	4.15 ± 0.01
	44.5	4.34 ± 0.02	4.29 ± 0.02	4.22 ± 0.02	4.14 ± 0.02	4.04 ± 0.02
	49.5	4.32 ± 0.03	4.27 ± 0.03	4.21 ± 0.03	4.13 ± 0.03	4.03 ± 0.03
	54.5	4.22 ± 0.03	4.18 ± 0.03	4.13 ± 0.03	4.07 ± 0.03	3.99 ± 0.03
HTris^{+}	25.4	8.15 ± 0.01	8.13 ± 0.01	8.11 ± 0.01	8.08 ± 0.01	8.04 ± 0.01
	30.4	7.94 ± 0.01	7.93 ± 0.01	7.93 ± 0.01	7.92 ± 0.01	7.90 ± 0.01
	35.0	7.90 ± 0.02	7.87 ± 0.02	7.84 ± 0.02	7.80 ± 0.02	7.75 ± 0.02
	39.8	7.81 ± 0.02	7.79 ± 0.02	7.77 ± 0.02	7.74 ± 0.02	7.70 ± 0.02
	44.5	7.77 ± 0.05	7.74 ± 0.05	7.71 ± 0.05	7.67 ± 0.05	7.61 ± 0.05
	54.5	7.64 ± 0.02	7.62 ± 0.02	7.59 ± 0.02	7.55 ± 0.02	7.50 ± 0.02
Benzoic acid	25.2	4.44 ± 0.03	4.64 ± 0.03	4.88 ± 0.03	5.15 ± 0.03	5.47 ± 0.03
	29.6	4.45 ± 0.03	4.65 ± 0.03	4.88 ± 0.03	5.14 ± 0.03	5.45 ± 0.03
	34.4	4.47 ± 0.03	4.67 ± 0.03	4.90 ± 0.03	5.17 ± 0.03	5.49 ± 0.03
	39.9	4.48 ± 0.03	4.66 ± 0.03	4.88 ± 0.03	5.12 ± 0.03	5.42 ± 0.03
	44.5	4.52 ± 0.03	4.71 ± 0.03	4.92 ± 0.03	5.17 ± 0.03	5.46 ± 0.03
	49.5	4.59 ± 0.02	4.78 ± 0.02	4.99 ± 0.02	5.24 ± 0.02	5.53 ± 0.02
	54.5	4.63 ± 0.02	4.80 ± 0.02	4.98 ± 0.02	5.20 ± 0.02	5.46 ± 0.02
Acetic acid	25.2	4.97 ± 0.03	5.14 ± 0.03	5.34 ± 0.03	5.57 ± 0.03	5.84 ± 0.03
	30.5	4.99 ± 0.02	5.16 ± 0.02	5.35 ± 0.02	5.57 ± 0.02	5.83 ± 0.02
	34.7	4.98 ± 0.02	5.13 ± 0.02	5.31 ± 0.02	5.52 ± 0.02	5.76 ± 0.02
	39.8	5.04 ± 0.02	5.19 ± 0.02	5.37 ± 0.02	5.58 ± 0.02	5.83 ± 0.02
	44.5	5.14 ± 0.07	5.29 ± 0.07	5.47 ± 0.07	5.67 ± 0.07	5.90 ± 0.07
	49.5	5.17 ± 0.03	5.32 ± 0.03	5.49 ± 0.03	5.69 ± 0.03	5.92 ± 0.03
	54.5	5.13 ± 0.05	5.30 ± 0.05	5.48 ± 0.05	5.70 ± 0.05	5.95 ± 0.05

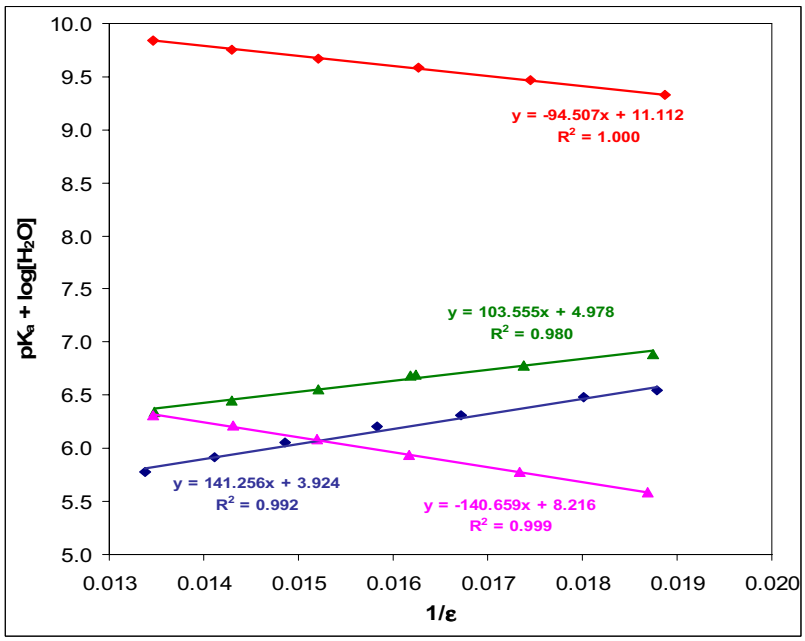

Figure 1.3.3 Variation of term pK_a + log [H_2O] from studied compounds vs 1/ε at 25°C ◆ Protonated tris, ▲ Acetic acid, ◆ Benzoic acid and ▲ Anilinium

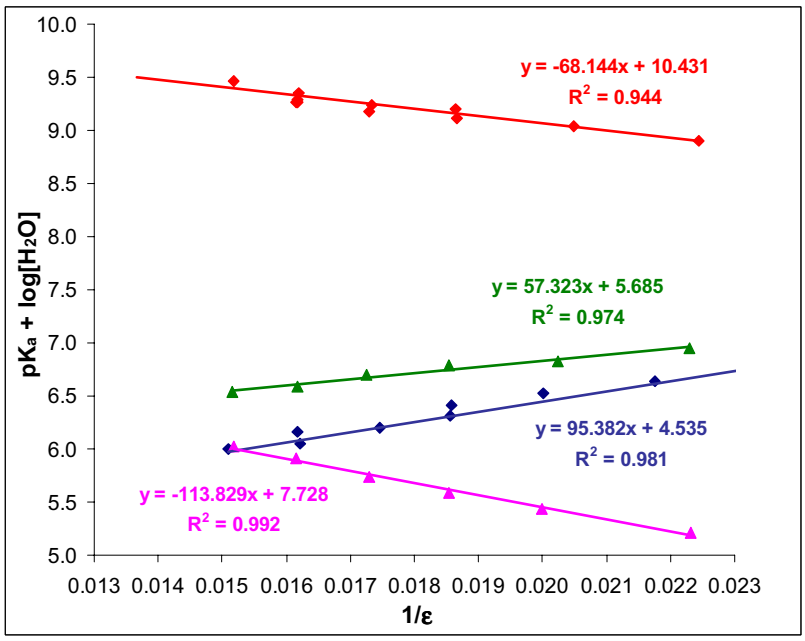

Figure 1.3.4 Variation of term pK_a + log [H_2O] from studied compounds vs 1/ε at 50°C ◆ Protonated tris, ▲ Acetic acid, ◆ Benzoic acid and ▲ Anilinium

1.3.4 Van't Hoff equation and determination of the enthalpy variation for studied compounds

Fig. 1.3.5 shows the ${}^{s}_{s}pK_a$ variation with temperature of the studied compounds. In all instances they could be fitted to a straight line and then the Van't Hoff equation [1.3.8] can be applied.

$$\ln(K) = -\frac{\Delta H^o}{R}\left(\frac{1}{T}\right) + \frac{\Delta S^o}{R} \qquad [1.3.8]$$

Therefore, the variation of the dissociation enthalpy, ΔH_{VH}, at the 25-55°C temperature range can be taken as a constant that can be easily calculated from the Van't Hoff slopes. Values of ΔH_{VH} are gathered in Table 1.3.5 for solutions from 0 to 50% of MeOH. The results show that the absolute ΔH_{VH} values for anilinium and HTris+ are significantly higher than those of benzoic and acetic acids and the dissociation process is exothermic for the cationic acids whereas it is slightly endothermic for the neutral acids in the solvent mixtures studied. In addition calculated ΔH_{VH} are close to those given in the literature and also to those determined from the pK_a values obtained by isothermal titration calorimetry (ITC) in pure water and in 50% methanol/water binary solvent. Table 1.3.7 also shows the ΔH_{VH} mean values determined directly by ITC in the 15-65°C temperature range as well as those given in literature. The consistency of the whole set of obtained values with those from literature allows us to conclude that the variation of enthalpy associated to an acidic dissociation process in methanol/water mixtures can be estimated from the thermodynamic dissociation constants determined in the 25-55°C temperature range.

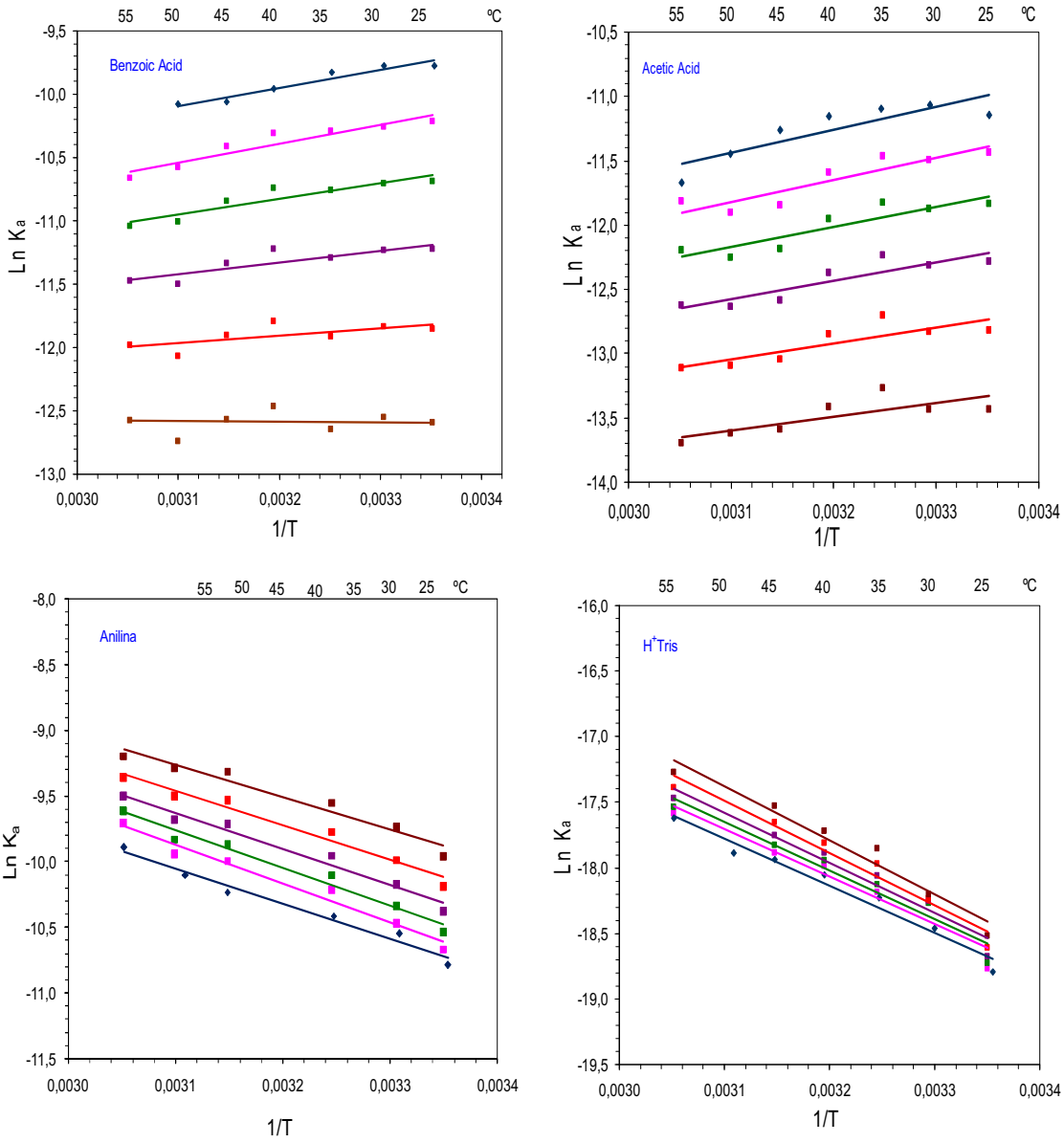

Figure 1.3.5 Van't Hoff plots and thermodynamic potentiometric pK_a values for Benzoic acid, Acetic acid, Anilinium and $HTris^+$ at various MeOH/H_2O mixtures: (♦) 0%, (■) 10%, (■), 20%, (■) 30%, (■) 40% and (■) 50% (w/w) in MeOH.

Table 1.3.7 Variation of enthalpy of several acidic dissociation constants

		From potentiometric pK_a values	From ITC pK_a values	From ITC in the Temp (15-55°C)	Literature values[d]
	MeOH% (w/w)	ΔH_{VH} (Kcal/mol)	ΔH_{VH} (Kcal/mol)	ΔH_{VH} (Kcal/mol)	ΔH_{VH} (Kcal/mol)
Anilinium	0[a]	5.3 ± 0.4	6.3 ± 0.2	6.67 ± 0.08	5.18 to 7.44
	10[b]	5.9 ± 0.5			
	20[b]	5.7 ± 0.5			
	30[b]	5.5 ± 0.5			
	40[b]	5.2 ± 0.5			
	50[b]	4.9 ± 0.6	5.2 ± 0.6	6.51 ± 0.12	---
Tris	0[a]	7.1 ± 0.6	---	10.74 ± 0.38	11.33 to 11.89
	10[b]	7.2 ± 0.9			
	20[b]	7.4 ± 0.9			
	30[b]	7.6 ± 0.8			
	40[b]	7.9 ± 0.8			
	50[b]	8.2 ± 0.8	---	12.10 ± 0.40	11.43
Benzoic Acid	0[a]	-2.8 ± 0.4	-1.6± 0.2	-0.27 ± 0.01	0.96 to -1.15
	10[b]	-2.9 ± 0.4			
	20[b]	-2.4 ± 0.4			
	30[b]	-1.9 ± 0.5			
	40[b]	-1.2 ± 0.6			
	50[b]	0.1 ± 0.4	0.1 ± 0.5	0.39 ± 0.04	0.7
	50[c]	0.15 ± 0.09			
Acetic Acid	0[a]	-3.5 ± 0.9	---	---	0.27 to -1.16
	10[b]	-3.4 ± 0.7			
	20[b]	-3.1 ± 0.7			
	30[b]	-2.8 ± 0.7			
	40[b]	-2.5 ± 0.7			
	50[b]	-2.1 ± 0.8	-2.2 ± 0.6	-0.08 ± 0.09	0.15 to -0.05

[a]Experimentally determined in this work, [b]Interpolate from the Yasuda-Shedlovsky plots built from experimental potentiometric pK_a's, [c]from Reference 28, [d]from reference 1

The results achieved clearly show that the four parameter calibration procedure can be applied to get accurate pK_a values in pure water and in methanol/water mixtures at the temperature range of 25-55°C. Moreover, the Yasuda-Shedlovsky equation and the calculated Debye-Hückel parameters allow the calculation of the thermodynamic acidity constants at any solvent composition (from 0 to 60% (w/w) of methanol) and temperature (from 25 to 55°C). The potentiometric results achieved agree with those from literature determined by means of calorimetric titrarions (ITC) when the protonation enthalpy is high enough. However, when small amounts of heat, lower than 1 kcal mol^{-1}, are involved in the protonation process slight differences in pK_a values achieved by both techniques are obtained. It has also been demonstrated that, at least for the studied compounds, the Van't Hoff equation is able to estimate the values for the enthalpy variation of the acidic dissociation processes.

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CHAPTER 2

Solubility of various bioactive compounds

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2.1 INTRODUCTION

Solubility is a key physicochemical property of bioactive compounds. An accurate measurement of the instrinsic solubility is important for many processes, but it is of special relevance in the pharmaceutical industry. Indeed, solubility plays a main role in the absorption and other pharmaceutical properties of drugs. So it must be addressed during the early stages of drug discovery to avoid the development of candidates with inadequate solubility under physiological conditions [1]. Different methods have been described to measure the solubility, the classical shake-flask (S-F) approach being the standard one. However, a common drawback in solubility measurement procedures is that they are tedious and time consuming. In addition, aqueous solubility is difficult to predict and computational models often show high uncertainties. Recently, *Sirius Analytical Instruments Ltd*. developed a new methodology, the Chasing Equilibrium method, to measure both kinetic and intrinsic solubilities. This is a highly reproducible potentiometric technique where the thermodynamic equilibrium is rapidly reached [2, 3]. The main requirement to measure solubility by this approach is that the compound must contain ionizable groups, acidic or basic, with known acidity constants. However, there are optimal experimental conditions in which measurements must be performed. The purpose of the present work is to test and validate this methodology by means of the determination of the solubility values of several bioactive compounds, including both acidic and basic compounds. Moreover, to propose a simple way to estimate the optimized amount of sample to get reliable solubility values, is a relevant goal of this investigation. This item is of practical interest because, commonly, only small amount of compound are available in the Drug Discovery step. Then, to be able to foresee the suitable sample weight should be a very useful tool in pharmaceutical laboratories.

2.1.1 Solubility measurement

2.1.1.1 Definitions

Solubility, S: solubility in water of a substance is specified by the saturation mass concentration of the substance in water. For ionizable compounds, solubility in a particular *pH* is defined as the sum of the concentration of all species dissolved in the aqueous solutions.

Intrinsic Solubility, S_o: is the solubility of the compound in its free acid or free base form. It is also called thermodynamic solubility of the neutral compound and it doesn't depend on *pH.*

Kinetic Solubility, S_K: is the solubility of the compound at the time when an induced precipitate first appears in a solution.

Supersaturated solution: is one in which the concentration of dissolved neutral species is greater than the intrinsic solubility. This is not at equilibrium and may or may not have solid precipitate present. If there is no solid present, then it will start to precipitate after a period of time. If precipitate is present, then the neutral species will continue to precipitate until eventually equilibrium is achieved.

Subsaturated solution: is one in which the concentration of dissolved neutral species is less than the intrinsic solubility. If there is precipitate present, then solution and the solid will not be in equilibrium and the solid will gradually dissolve until eventually equilibrium is achieved or the solid is entirely dissolved.

2.1.1.2 Potentiometric methods

Despite the classical method for solubility determination, the Shake-Flask one [4], here we use a potentiometric approach. The potentiometric method described by Avdeef for the first time is a good alternative for thermodynamic measurements of the solubility of the neutral form of ionizable compounds, the intrinsic solubility [5]. The method involves titrating a basic compound from high to low *pH*, or an acidic compound from low to high *pH*, and calculating the apparent ionization constant from the *pH* of each point in the full titration curve. The intrinsic solubility is calculated from the shift of apparent ionization constant compared to the aqueous ionization constant. The *pH* in regions of the titration curve where precipitated neutral compound is present should be measured under equilibrium conditions. A typical titration takes 3−10 hours to complete dissolution as the time taken to dissolve additional solid increases significantly at this point. From calculated solubility and pK_a, a solubility-*pH* profile is obtained [6].

A new potentiometric approach, called Chasing Equilibrium, has been developed for measuring the intrinsic solubility of ionizable compounds [2]. In this method, a solution of the drug in ionized form is automatically titrated with acid or basic titrant to convert the sample to neutral form. Eventually the sample precipitates. At this point, while the experiment is near equilibrium, the instrument adds small increments of KOH and HCl solution. This forces the neutral species to cycle between supersaturated and subsaturated states. Between these states, the experiment would be at equilibrium. Monitoring the *pH* changes allows the determination of equilibrium *pH*. From this, the intrinsic solubility of the compound is determined using appropriated mass and charge balance equations. The method also allows the kinetic solubility determination. It corresponds to the concentration of neutral concentration when the first precipitated appears. The process is relatively quick and can determine intrinsic solubilities in 20 – 80 min/sample [2, 7].

2.1.1.3 CheqSol method

The CheqSol method is the titrimetric method developed by Sirius based in Chasing Equilibrium approach. The method proposed consists in the titration of ionisable compound until precipitation begins. Then, the addition of titrant is stopped but the sample continued to precipitate in order to reach the equilibrium. In this stage, the *pH* is measured once per second and, as shown in Fig. 2.1.1 for a monoprotic base, a small decrease in *pH* is observed when it precipitates. Conversely, a small increase in *pH* is observed when a neutral acid precipitates. When the rate of change in *pH* becomes linear with time to better than 90%, the *pH* and the slope (d(*pH*)/dt) is recorded, and then small aliquots of KOH and HCl are added to 'flip' the system from supersaturated to subsaturated and back, each time recording *pH* and the slope when it reached a stable rate of change.

The direction of the *pH*-gradient gives an indication of the saturation level of the solution and allow to assess the direction toward equilibrium. For example, an unionized base precipitating from a supersaturated solution produce a negative *pH*-gradient because the neutral compound (B) lost from solution is replaced by more neutral compound, formed from the cations, whereas an unionized base dissolving from a subsaturated solution produce a positive *pH*-gradient. Then, the *pH* gradient is used to determine the type of titrant to be added to move the *pH* in equilibrium direction more quickly than it would have proceeded unaided. The rapid *pH* change due to titrant addition changed the ionization of the sample, altering the concentration of the neutral (unionized) form, and taking the system closer to, or beyond, equilibrium. When the system had been taken beyond equilibrium, the *pH* gradient reversed and Chasing Equilibrium then proceeded by adding the opposite titrant.

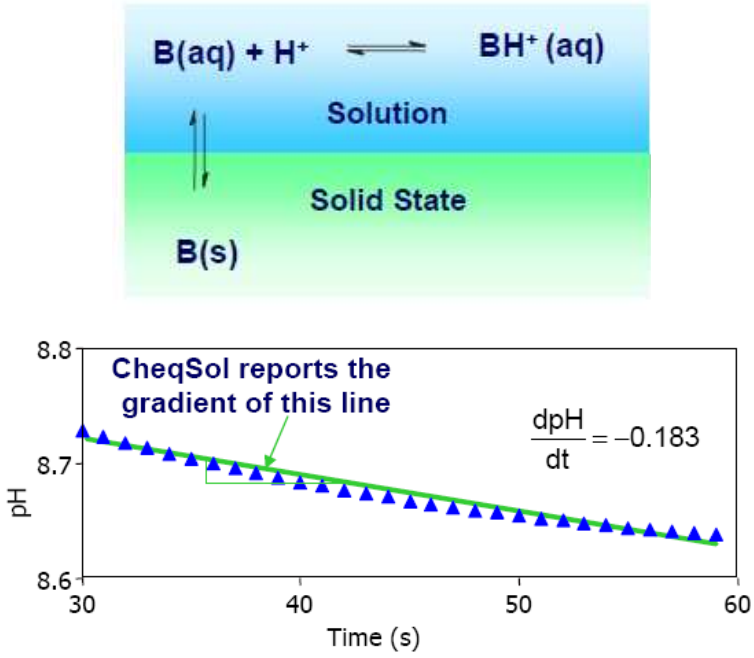
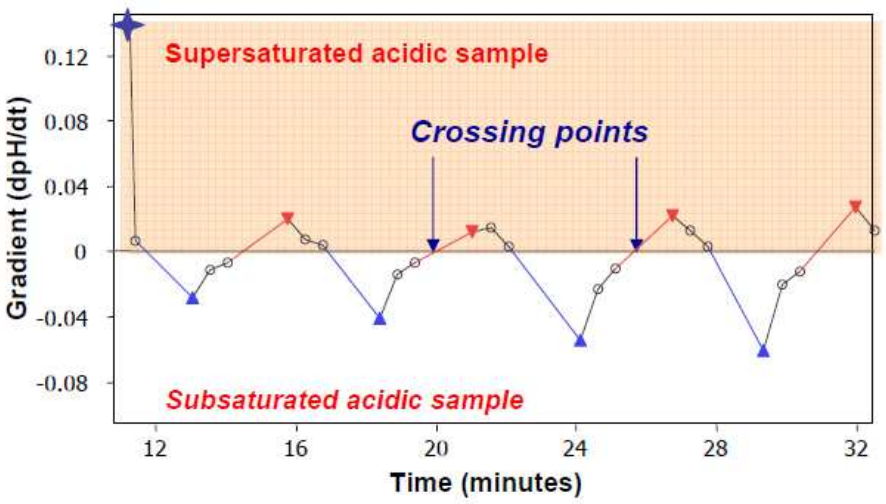


Figure 2.1.1: *pH*-gradient for monoprotic base

An analogous process occurs when the sample is a neutral acid.

Fig. 2.1.2 shows the plot of slope d(*pH*)/dt *vs*. time, this is, the change of *pH*-gradient while Chasing Equilibrium of a weak acid. According to this graph, the system would be at equilibrium at the crossing points, where the slope d(*pH*)/dt = 0. Then, the concentration of neutral species can be calculated at each point in the graph applying the principles of mass balance. Fig. 2.1.3, show the plot of the slop *vs.* concentration. It can observed that all crossing points fall on the d(*pH*)/dt = 0 axis at similar concentration, which turns out to be the intrinsic solubility of the weak acid. Then the intrinsic solubility is determined by average concentration of HA at these crossing points. The spread of these crossing points is used to determine the precision of the mean intrinsic solubility result [2, 3, 8].



Black lines and circles - nothing added

Blue lines and squares - KOH added

Red lines and triangles - HCl added

Figure 2.1.2: Gradient versus time plot for a neutral acid

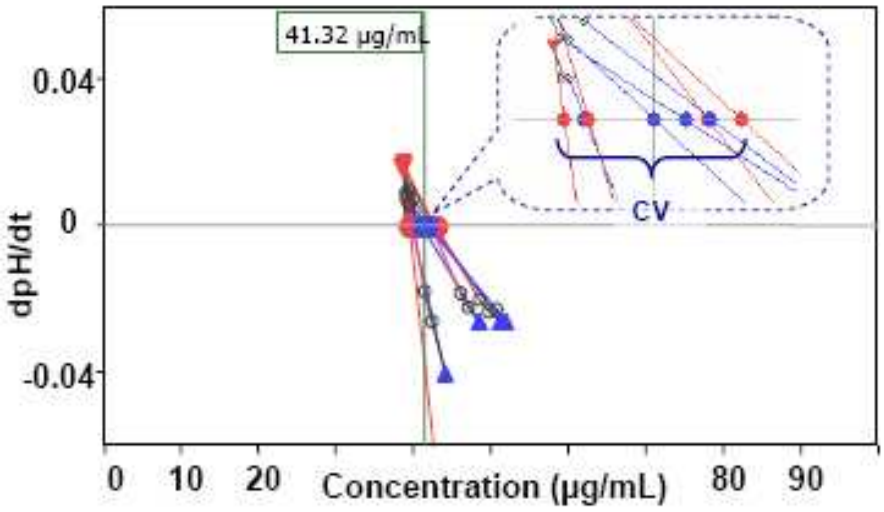


Figure 2.1.3: Concentration crossing point plot for a neutral acid

The experimentally method proceeded in five steps: dissolution, seeking precipitation, additional precipitation, Chasing Equilibrium, and redissolution [2].

Dissolution: A selected quantity of substance is accurately weighed into the titration vessel and dissolved in a measured volume of KCl solution. A measured volume of either acid or base titrant is added to adjust the solution to at *pH* at which the solute is fully dissolved in its ionized form. If the sample is an acid, the *pH* is adjusted by adding base titrant. If the sample is a base, acid titrant was added.

Seeking precipitation: The solution of ionized solute is back-titrated towards precipitation by adding measured aliquots of base or acid. The exact point of precipitation is recorded by spectroscopic dip probe (*D-PAS* probe), by examining the solution for evidence of light scattering caused by turbidity. The use of dip probe made possible to automate the solubility analysis, as there was no need for a person to watch the experiment.

Additional precipitation: Additional aliquots of the same titrant were added, until the *pH* had changed by a further predefined increment (e.g., 0.1 *pH* unit) or until a fixed time had elapsed (e.g., 60 s) should the precipitation cause the *pH* to spontaneously readjust as quickly as titrant was added. The purpose of the additional precipitation stage was to ensure that sufficient precipitation was present for the next stage of the experiment.

Chasing Equilibrium: When precipitation of the neutral species happens, the solution is repeatedly changed from supersaturated to subsaturated and back again by changing the *pH*. This stage is repeated until around eight saturation state changes are measured.

Redissolution: After sufficient data has been collected to calculate a solubility result, the *pH* is adjusted to a value at which the sample becomes fully ionized and the solution was held at that *pH* while the sample dissolved. The purpose of this stage was to ensure that no crystals or solid sample remained on the apparatus that may have impaired its performance in subsequent assays. After redissolution, the probes were washed before any further actions took place.

A useful visualization of the method can be obtained from the Bjerrum function which shows the average number of bound protons ($\bar{n}$) versus *pH.* Fig. 2.1.4 show an example of Bjerrum plot for a neutral monoprotic acid, HA.

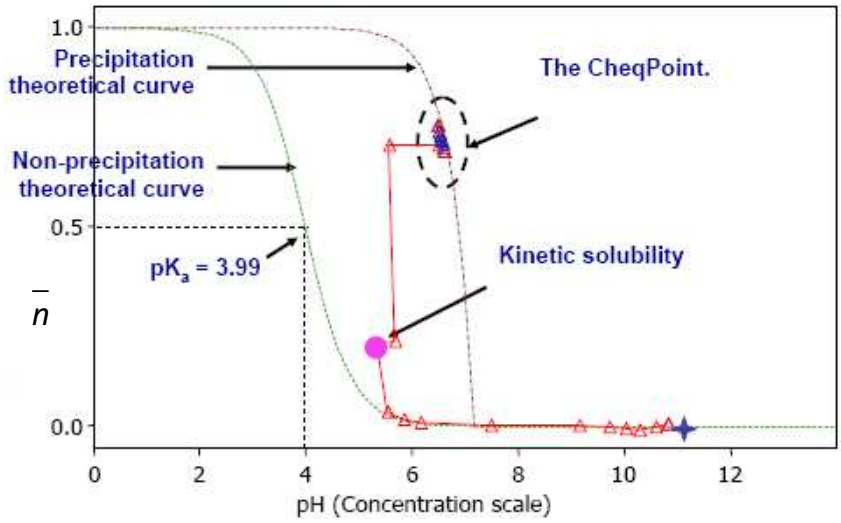


Figure 2.1.4: Bjerrum plot for a monoprotic acid

Two theoretical curves appear in this graph. The first one, indicated as green line, corresponds to the nonprecipitation theoretical Bjerrum curve which represents the hydrogen-ion binding capacity of the solute during the aqueous titration with no precipitate. This curve is obtained from the aqueous pK_a of the solute by means of Eq [2.1.1]

$$\bar{n} = \frac{[H^+]}{K_a + [H^+]} \qquad [2.1.1]$$

The second curve, indicated as brown line, corresponds to the precipitation theoretical curve which represent the hydrogen-ion binding capacity of the solute at equilibrium with precipitated solid. The curve is described by Eq [2.1.2]:

$$\bar{n} = 1 - \frac{S_o K_a}{C_a [H^+]} \qquad [2.1.2]$$

For monoprotic bases, again Eq. [2.1.1] is used to describe the theoretical Bjerrum functions in absence of precipitate. Eq. [2.1.3] describes the theoretical Bjerrum functions for a monoprotic base in presence of precipitate.

$$\bar{n} = \frac{S_o \left[H^+ \right]}{C_a K_a} \qquad [2.1.3]$$

The distance between the two theoretical curves depends on the solubility of the substance: the lower the solubility, the greater the distance is.

For a monoprotic acid, HA, while there is an absence of the precipitate (dissolution and seeking precipitation steps) the experimental data must be fit well to the nonprecipitation theoretical curve (see Figure 2.1.4). When a precipitate appears, the experimental points do not follow this theoretical curve any more. The *pH* corresponding to the moment when precipitation is first observed is known as the Precipitation Point (marked in the plot as a pink point) and indicates the kinetic solubility. Here *pH* slightly increase because of the HA precipitation. This point is close to the nonprecipitated Bjerrum curve. Further addition of acidic titrant leads to a higher concentration of HA and *pH* increase. On the Chasing Equilibrium step the solution is close to equilibrium with the precipitate, then, data points collected during this period lie close together on the precipitation theoretical curve at a point known as the CheqPoint [2, 3, 8].

2.2 EXPERIMENTAL

2.2.1 Potentiometric determination of Solubility using PCA200 analyzer

2.2.1.1 Instrument

The instrument used to perform solubility determination was PCA200 titrator and D-PAS spectrometer controlled from a computer running RefinementPro and CheqSol software which has been developed by Sirius Analytical Instruments Ltd. (shown in Fig. 2.2.1) to measure pK_a, log *P* and determine kinetic and intrinsic solubility of ionizable compounds [7].

Figure 2.2.1: PCA200

A schematic of the titration head is shown in Fig. 2.2.2. The *pH* electrode, KFP-0693 (Combination Ag-AgCl), was connected to custom-designed *pH* sensing circuit ($10^{15}\,\Omega$ impedance) for measuring *pH* and calibrated titrimetrically in the *pH* range 1.8−12.2.

An over head stirrer was connected to a motor whose speed of rotation was controlled by the computer program. The dispenser tips were made from narrow polyimideclad quartz capillary (0.5 mm inside diameter) tubes that were connected to precision dispensers that were able of delivering small reproducible aliquots of liquid of known volume shown in the Fig. 2.2.3. The temperature probe monitored the temperature during the period of the titration. A difurcated fiber-optic probe (Hellma) with path length of 1 cm was connected to a *D-PAS* ultraviolet spectrometer. Also we have argon cap which is bubbled through the water and base titrant in order to reduce the effect of CO_2 adsorption during titration. A degasser is also needed to remove any carbon dioxide from reagents.

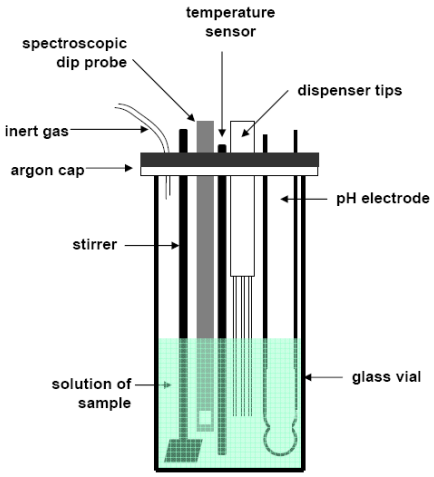


Figure 2.2.2: Measurement cell – set up in temperature-controlled sample holder on PCA200

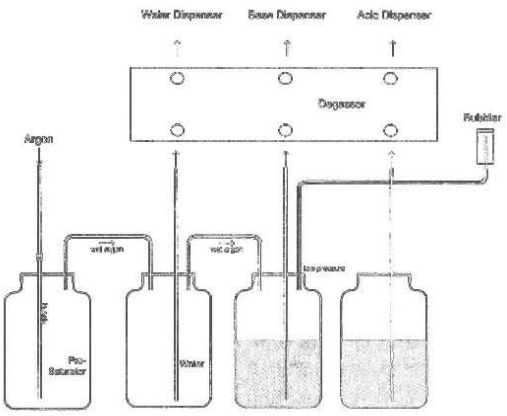


Figure 2.2.3: Regent Tubing Schematic

2.2.1.2 Reagents and solvents

- Buffer solution *pH* 7.00, Crison
- Hydrochloric acid 1 M, Merck, Titrisol®;
- Potassium hydroxide 1 M, Merck, Titrisol®;
- Potassium chloride, Merck, > 99.5%
- Potassium biphtalate, Merck, > 99.8%

- Potassium dihydrogen phosphate (KH_2PO_4), Merck > 99.5%
- Methanol, Merck, HPLC grade
- Water purified by a Milli-Q plus system from Millipore with resistance higher than 18MΩ

2.2.1.3 Studied Substances

Acids:

- Methyl paraben (Methyl 4-hydroxybenzoate), Fluka ≥ 99% (GC)
- Ethyl paraben (Ethyl 4-hydroxybenzoate), Aldrich ≥ 99%
- Propyl paraben (Propyl 4-hydroxybenzoate), Fluka ≥ 99% (GC)
- Butyl paraben (Butyl 4-hydroxybenzoate), Fluka ≥ 99% (GC)
- Warfarin ((*RS*)-4-hydroxy- 3-(3- oxo- 1-phenylbutyl)- 2*H*- chromen- 2-one), Sigma > 98%

Parabens are labile esters that can experiment alkaline hydrolysis at high *pH* values. As solubility experiments for these compounds are performed at basic *pH*s (*pH*=12) for the redissolution step, and *pH*≈10 or lower for the seeking step, the stability of the parabens at these *pH* values was examined. The stability experiments were performed for a time period higher than the one required in the solubility experiments, and no anomalous behavior attributable to hydrolysis processes was observed.

Bases:

- Methyl 4-aminobenzoate, Aldrich > 98%
- Benzocaine (Ethyl 4-aminobenzoate), Fluka > 99%
- Butamben (Butyl 4-aminobenzoate), Fluka > 98%
- Lidocaine ([2-Dietylamino-N-C2, 6-dimethylphenyl]-acetamide), Sigma > 98%

2.2.1.4 Procedure

2.2.1.4.1 Standardization parameters at 25°C

The same process mentioned in sections 1.2.4.1 and 1.2.4.2 (chapter 1) to standardize the system has been used.

2.2.1.4.2 Potentiometric determination of pK_a values in aqueous solution at 25°C, ${}^{w}_{w}pK_a$

Before measuring solubility, pK_a value of methylparaben was determined using the same process described in section 1.2.4.3 (Chapter 1). Experiments were carried out with the PCA200 apparatus. A minimum of three measurements for each compound were carried out, and the pK_a values, ${}^{w}_{w}pK_a$, were calculated through the RefinementPro software.

2.2.1.4.3 Potentiometric determination of pK_a values in several mixtures of methanol/water (0−60% w/w) at 25°C, ${}^{s}_{s}pK_a$, and ${}^{w}_{w}pK_a$ calculation

The ${}^{s}_{s}pK_a$ of various compounds poorly soluble in pure water like ethylparaben, butylparaben, lidocaine and propylparaben were measured in various methanol-water mixtures (from 10 to 60 weight % of methanol). In order to have accurate results before measuring solubility, ${}^{s}_{s}pK_a$ values of these studied compounds were determined using the same process described in section 1.2.4.3 (Chapter 1). Experiments were carried out with the PCA200 apparatus. ${}^{w}_{w}pK_a$, were extrapolated from different ${}^{s}_{s}pK_a$ values determined at different mixtures of methanol/water through the RefinementPro software.

2.2.1.4.4 Spectrophotometric determination of pK_a values in aqueous solution at 25°C, ${}^{w}_{w}pK_a$

The determination of the ${}^{w}_{w}pK_a$ values of methyl 4-aminobenzoate, benzocaine, butamben and warfarin was carried out by spectrophotometry. These experiments were also carried out with the PCA200 apparatus. A minimum of three measurements for each compound were carried out. Briefly, a 10 mM stock solution of sample was prepared in DMSO. 50 µL of sample stock solution and 0.25 mL of a 15 mM potassium phosphate buffer were added to 10 mL of a 0.15 M KCl solution. The *pH* of the sample solution was adjusted to 1.8 with 0.5 M HCl before starting the titration, and then the titration was done using 0.5 M KOH. The UV absorption spectra of the solution were continuously monitored in the titration vial by a fiber optic dip-probe. The collected data were refined through the RefinamentPro software and the ${}^{w}_{w}pK_a$ values obtained by Target Factor Analysis.

2.2.1.4.5 Solubility measurement and calculation

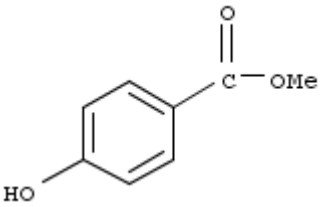
An amount of substance (>10 mg) was precisely weighted into the titration vessel and a measured volume (10 mL) of 0.15 M KCl solution was added. Then, the described five step procedure (section 2.2.1.3) begins. The occurrence of precipitation was detected using a spectroscopic dip probe (Fig. 2.2.4). The solubility is calculated by means of the CheqSol software.

Figure 2.2.4: dip probe-connected to D-PAS

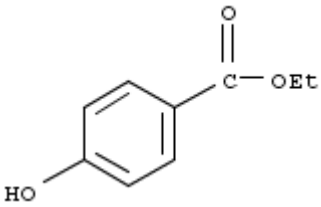
2.3 RESULTS & DISCUSSION

2.3.1. Studied compounds

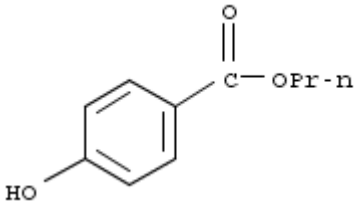
Some acidic and basic compounds have been chosen for this study. Acidic compounds under study have been chosen from paraben family including methylparaben, ethylparaben, propylparaben and butylparaben and also warfarin uniquely from different acidic family. Compounds like methyl 4-aminobenzoate, benzocaine and butamben from the same family and also lidocaine, uniquely from different basic family have been chosen as basic compounds. Structures of these substances are shown in Fig. 2.3.1.



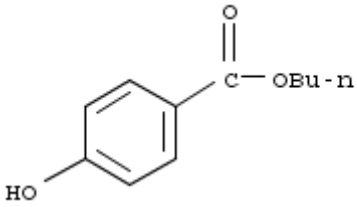
Methylparaben



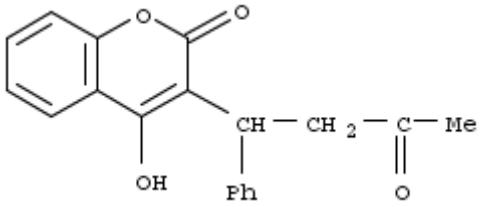
Ethylparaben



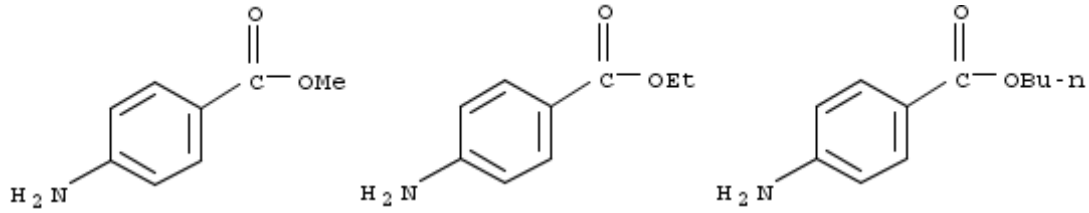
Propylparaben



Butylparaben



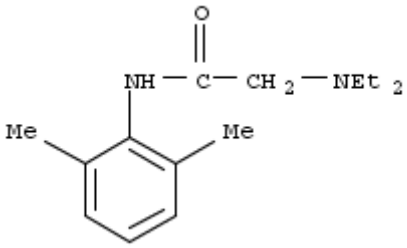
Warfarin



Methyl 4-aminobenzoate

Ethyl 4-aminobenzoate (Benzocaine)

Butyl 4-aminobenzoate (Butamben)



Lidocaine

Figure: 2.3.1 Structure of studied compounds

2.3.2 Aqueous pK_a values for studied compounds at 25°C

The value obtained for solubility directly depends on the pK_a value used in the calculations. In fact, each unit of error in the value used for the pK_a closest to the neutral species gives an error of the order of one logaritmic unit in the intrinsic solubility result [2]. It is therefore, important to use well-determined pK_a values when measuring solubility by Chasing Equilibrium method. Then the acidic dissociation constant values for the selected compounds were determined by means of PCA200 instrument under the same conditions of ionic strenght and temperature used in solubility determination. All measurements were performed in triplicate. In Table 2.3.1, the type of compound, method for pK_a determination and

aqueous pK_a values, ${}^{w}_{w}pK_a$, for all studied compounds are given. Since, henceforth all the pK_a values are referred to aqueous solutions, the symbol, ${}^{w}_{w}pK_a$ will turned into pK_a.

Table 2.3.1 Aqueous pK_a of studied compound at 25 °C

Compound	Type	Method	pK_a Exp(I=0.15)	pK_a Exp(I=0)	pK_a Lit
Methylparaben	Acid	Pot. Aqueous	8.15 ± 0.01[a]	7.90	8.47[b]
Ethylparaben	Acid	Pot.water/methanol	8.18 ± 0.02[a]	7.92	8.50[b]
Propylparaben	Acid	Pot.water/methanol	8.16 ± 0.04[a]	7.90	8.47[b]
Butylparaben	Acid	Pot.water/methanol	8.18 ± 0.04[a]	7.92	8.47[b]
Warfarin	Acid	D-PAS. Aqueous	4.88 ± 0.01	4.63	5.01[c]
Methyl 4-aminobenzoate	Base	D-PAS. Aqueous	2.66 ± 0.05	2.66	2.47[d]
Benzocaine	Base	D-PAS. Aqueous	2.67 ± 0.01	2.67	2.51[d]
Butamben	Base	D-PAS. Aqueous	2.56 ± 0.02	2.56	2.47[d]
Lidocaine	Base	Pot.water/methanol	7.98 ± 0.04[a]	7.98	7.96[e]

[a]from reference[13], [b]from reference [9], [c]from reference [12], [d]from reference [10], [e]from reference [11]

pK_a values of parabens are similar because all of them belong to the same family and they are in agreement with those one from literature. It is important to take into account that the values from literature are given at various ionic strenghts. The pK_a value obtained for warfarin is also very close to the one already reported. The pK_a values of methyl 4-aminobenzoate, benzocaine and butamben are also similar and consistent with those from literature. Finally, pK_a value obtained for lidocaine is in agreement with the published one.

2.3.3 Determination of the intrinsic and kinetic solubility

It is very important for pharmaceutical industry to be able to measure the solubility using the minimum weight of compound since, very often the amount of available sample is limited. Then, it is very important to know which weight range of the studied compound is the optimal one to achieve the best results. Hypothetically, the optimal sample weight to perform a solubility measurement through this method would be the one corresponding to $\bar{n}_{CheqPoint}$ = 0.5. In order to explain this behavior, Fig. 2.3.2 A shows the Bjerrum plots for a monoprotic acid, propylparaben, when $\bar{n}_{CheqPoint}$ = 0.5. It was observed a very good fitting of the experimental points to the theoretical curve, the green one, when $\bar{n}_{CheqPoint}$ = 0.5.

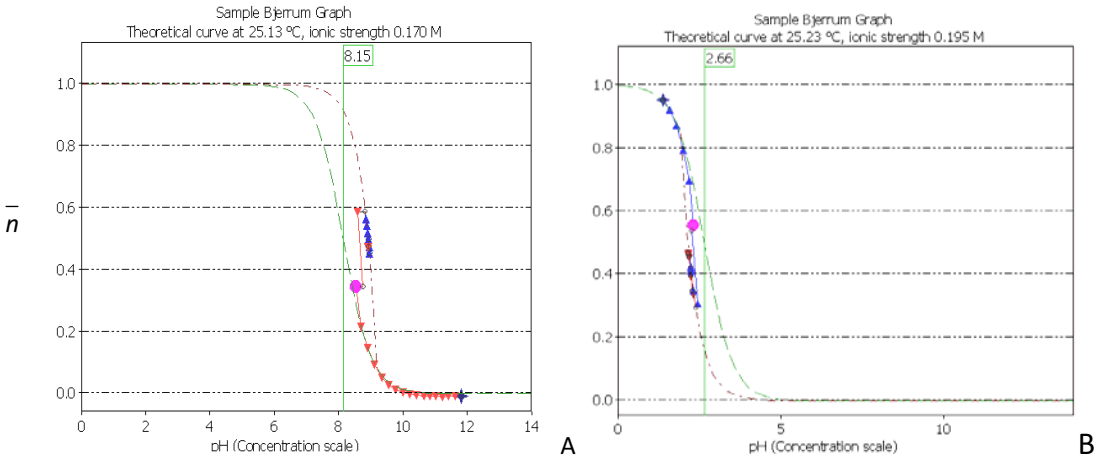


Figure 2.3.2 A) Bjerrum graph for propylparaben as a acid when $\bar{n}$ = 0.5 and weight=0.044 g B) Bjerrum graph for methyl 4-aminobenzoate as a base when $\bar{n}$ = 0.45 and weight=0.1 g

Fig. 2.3.2 B shows the Bjerrum plots for a monoprotic base, 4-aminobenzoate when $\bar{n}_{CheqPoint}$ = 0.45. It was also observed a very good fitting of the experimental points to the theoretical curve, the green one, when $\bar{n}_{CheqPoint}$ = 0.45. It is shown that in the case of bases the theoretical green curve moves to the left side of the red curve.

It is strongly recommended to keep the number of bound protons in the CheqPoint between 0.2 and 0.8. The reason is that the fraction of both ionic and neutral species must be high enough when Chasing Equilibrium takes place. In case of monoprotic acids, a $\bar{n}_{CheqPoint}$ value lower than 0.2 implies that less than 20% of the acid is in the neutral form (HA), whereas $\bar{n}_{CheqPoint}$ higher than 0.8 means that less than 20% of the acid is in the deprotonated form (A^-). The same reasoning applies to monoprotic bases, although now $\bar{n}_{CheqPoint}$ values lower than 0.2 mean that less than 20% of the base is in its ionic form (BH^+), whereas $\bar{n}_{CheqPoint}$ values higher than 0.8 imply that less than 20% of the base is in its neutral form (B). A compromise where $0.2 < \bar{n}_{CheqPoint} < 0.8$ ensures enough amount of both species to perform reliable solubility determinations. Table 2.3.2 shows the S_o and S_k values for the nine studied compounds, together with the standard deviation (SD) and the relative standard deviation (RSD). Also literature values of S_o are given. The solubility values, are the mean ones obtained from at least five replicates.

Table 2.3.2 Intrinsic (S_o) and kinetic (S_k) solubility values of studied compounds by Chasing Equilibrium method

Compound	S_o(μg/mL)	RSD (%)	S_o lit (μg/mL)	S_k (μg/mL)	RSD (%)
Methylparaben	2207 ± 100[a]	4.5	2130 ± 120[b]	4220 ± 1150[a]	27.3
Ethylparaben	921 ± 26[a]	2.8	1160 ± 210[b]	1887 ± 442[a]	23.4
Propylparaben	336 ± 8.3[a]	2.5	370 ± 30[b]	911 ± 313[a]	34.4
Butylparaben	217 ± 9.0[a]	4.1	158 ± 14[b]	440 ± 22[a]	4.9
Warfarin	5.6 ± 0.4	7.1	4.8 ± 0.3[c]	106 ± 8	7.1
Methyl 4-aminobenzoate	1186 ± 60	5.0	-	1766 ± 373	21.1
Benzocaine	779 ± 52	6.7	780[d]	1141 ± 269	23.6
Butamben	165 ± 7	4.5	-	282 ± 72	25.6
Lidocaine	3082 ± 94[a]	3.1	3500 ± 100[e] 3810 ± 222[f]	3865 ± 772[a]	20

RSD is expressed in %., [a]From reference [13], [b]From reference [14], [c]From reference [17], [d]From reference [16], [e]From reference [2], [f]From reference [15]

RSD for S_o are lower than 10% which is common in solubility measurements (EPA guideline recommends RSD lower than 30% for the shake-flask method) [4]. As expected, for the homologous series of parabens, the lower S_o value corresponds to butylparaben, which is the one with longer alkylic chain. When these values are compared to the ones given by literature (shake-flask method) good concordance is observed, despite literature results are from experiments performed in pure water and our are performed also in water but the ionic strength is 0.15 M. Solubility value for warfarin also is in a good agreement with the one from literature. Also for the homologous series of 4-aminobenzoate, the lower S_o value corresponds to butamben, which is the one with longer alkylic chain. In this series, when the solubility value of butylparaben, is compared to the one given by literature good concordance is observed but there is a lack of published values for other family members. Solubility value of lidocaine is also in a good agreement with the one from literature.

The Chasing Equilibrium method also provides the kinetic solubility (S_k). Table 2.3.2 shows these values, together with the SD and the RSD, too. As expected, in all cases S_k are higher than S_o values. It is also observed that RSD are also higher than the ones for S_o measurements. However, all of them are lower than 30%. This larger dispersion is attributed to the fact that S_k values are highly dependent on experimental conditions, such as the speed of titration (which affects the degree of supersaturation before precipitation begins), stiring velocity and the sensitivity of the precipitation detector [2], which produces the highest standard deviation around the mean value. In addition, kinetic solubility values are not thermodynamic measurements, and the precipitates formed at this point are kinetically driven. Repeated experiments often lead to a large SD around the mean value for S_k. When isolation of these first precipitates is possible, solid-state studies show that these precipitates are, in most cases, a different polymorphic form to the precipitates isolated at equilibrium [18]. The differences in precipitate polymorphism, which depend on whether precipitation is kinetically or thermodynamically driven [19], also explain the discrepancies between S_o and S_k values. The kinetic solubility, then, can not be used as a reliable guide to the intrinsic solubility of a compound.

Although S_k measurements are in most cases preferred to S_o measurements, since they are easier to perform, it is clearly demonstrated that they are much less accurate.

2.3.4 Effect of sample weight on solubility determination

In order to evaluate the efect of sample weight on solubility measurements, the solubility experiments were performed at different sample weights for all studied compounds. First, lidocanie and warfarin as a basic and acidic model drugs were studied. Then paraben family were studied as acidic drugs and also 4-aminobenzoate family have been chosen as basic drugs.

2.3.4.1 Warfarin and lidocaine as single models of monoprotic acids and bases

Warfarin and lidocaine have been chosen as single models of monoprotic acids and bases for our study. As the pK_a value is very important in solubility measurement, the pK_a values of these compounds have been measured (Table 2.3.1). The working *pH* is between 1.8-12 and the pK_a values of warfarin and lidocaine are in the central region of this *pH* range.

Numeric results obtained from different amounts of sample are given in the Appendix (Tables (2-1A), (2-2A)). Results for warfarin and lidocaine are shown in Fig. 2.3.3 and the Table 2.3.2. Warfarin has a S_o value of 5.6 ± 0.4 μg/mL and lidocaine 3082 ± 94 μg/mL; standard deviations (SD) increased according to the magnitude of S_o value.

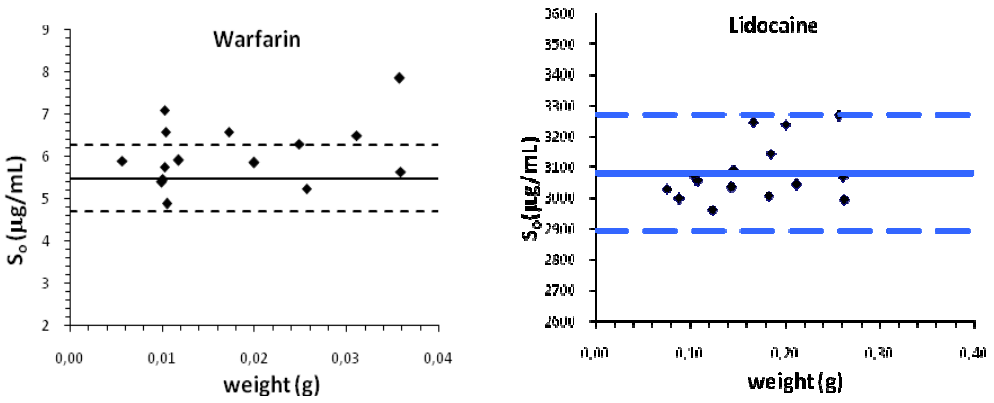


Figure. 2.3.3 Intrinsic solubility (µg/mL) vs. sample weight (g) plots of the warfarin and lidocaine Dashed lines correspond to S_o ± 2SD, and the solid line to the S_o mean value.

In Fig. 2.3.3, the average S_o value is indicated with a solid line, and the values of S_o ± 2SD with dashed lines. Most experimental values are randomly distributed in the S_o ± 2SD range. Some outliers are observed in the case of warfarin, when both the lowest or the highest amounts of drug were weighted. These results show that a well defined weight of sample range is associated to each compound.

2.3.4.2 Paraben and 4-aminobenzoate families

The same evaluation has been done for paraben and 4-aminobenzoate families. Numeric results are given in the Appendix (Tables (2-3A)-(2-6A), (2-7A)- (2-9A)). Results for paraben family are shown in Fig. 2.3.4 and for 4-aminobenzoate family in Fig. 2.3.5. As expected for both series, their solubility increased as the alkyl chain length decreased and the standard deviations (SD) increased according to the magnitude of S_o value. The outliers appear at the lowest or the highest weights of sample in both instances pointing out the suitable working limits.

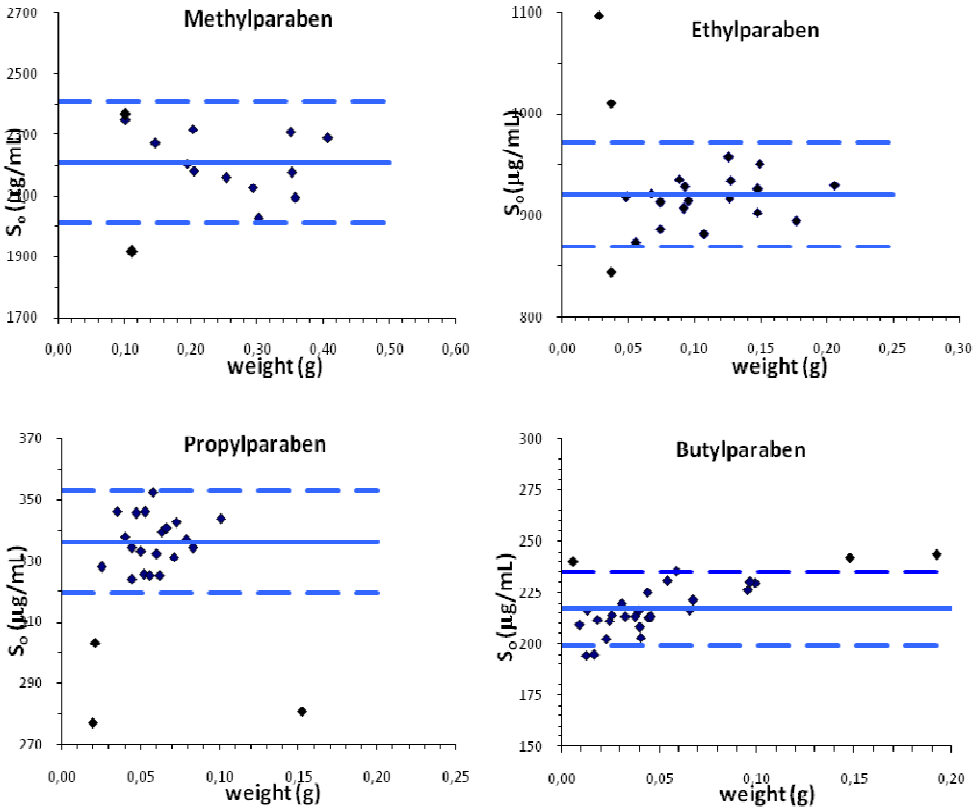


Figure. 2.3.4. Intrinsic solubility (µg/mL) vs. sample weight (g) plots of the paraben family. Dashed lines correspond to $S_o \pm 2SD$, and the solid line to the S_o mean value.

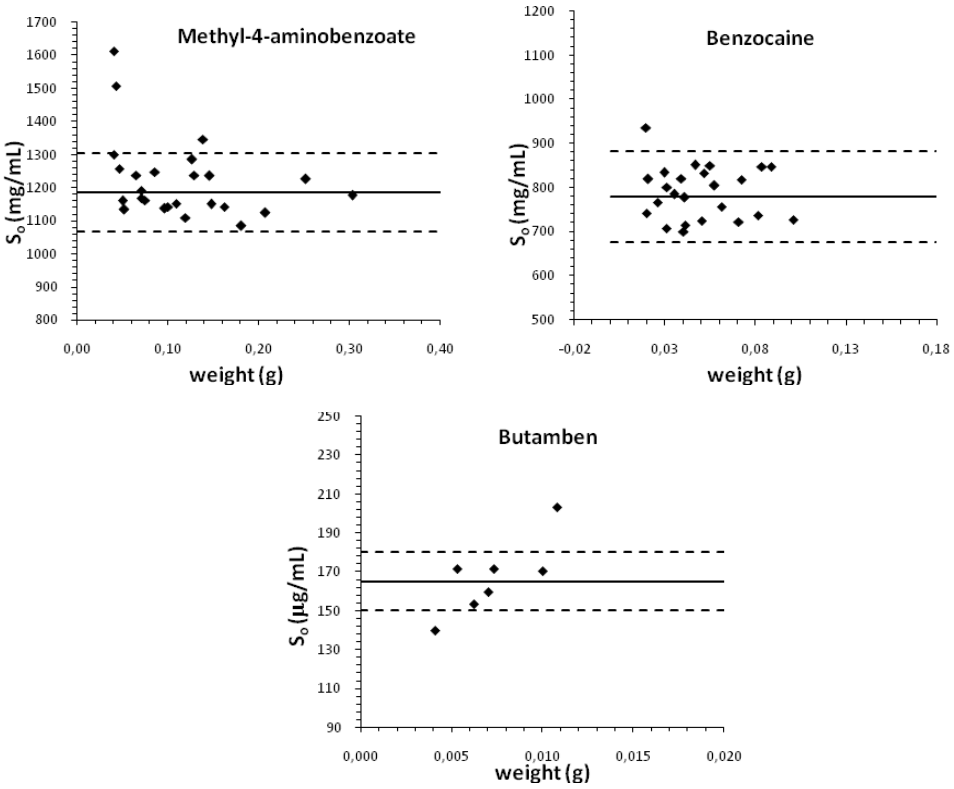


Figure. 2.3.5 Intrinsic solubility (µg/mL) vs. sample weight (g) plots of the 4-aminobenzoate family. Dashed lines correspond to S_o ± 2SD, and the solid line to the S_o mean value.

2.3.5 Evaluation of $\bar{n}_{CheqPoint}$ with sample weight

It is observed that the $\bar{n}$ value corresponding to experimental CheqPoint ($\bar{n}_{CheqPoint}$) changes with sample weight. In order to explain this behaviour here are shown two Bjerrum plots for propylparaben at both, the lowest and the highest sample weight (Figs. 2.3.6, 2.3.7).

Intrinsic solubility is defined as the concentration in solution of the neutral form of the compound in equilibrium with its precipitate. Therefore, for a neutral acid the higher is the sample weight, the faster the solubility value will be reached when lowering the *pH*. i.e., precipitation occurs at higher *pH* values. As a consequence,

precipitate is obtained at low $\bar{n}$ values (Fig. 2.3.6). On the contrary, when sample weight is low, solubility value reached at lower *pH* values, that implies higher $\bar{n}$ (Fig. 2.3.7).

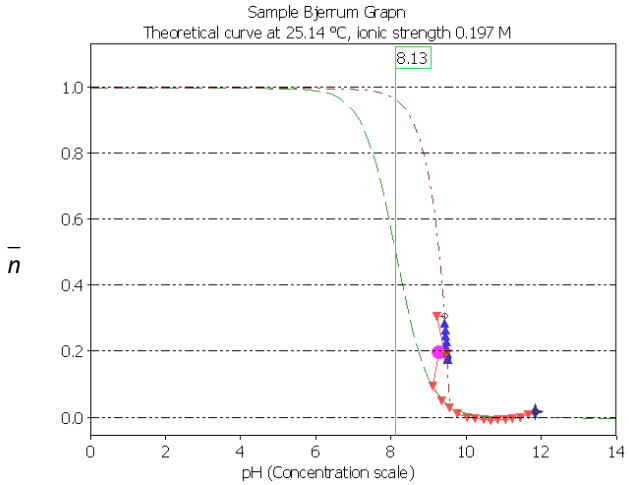


Figure 2.3.6 Bjerrum graph for propylparaben when $\bar{n}$ = 0.2 and weight=0.1222 g

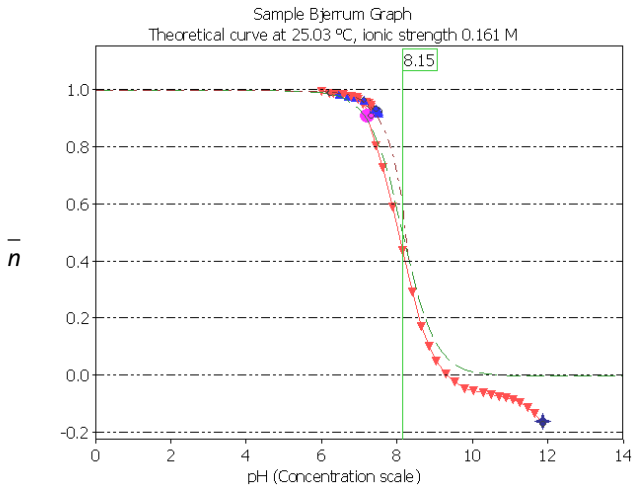


Figure 2.3.7 Bjerrum graph for propylparaben when $\bar{n}$ = 0.9 and weight=0.0144 g

2.3.5.1 Parabens family as acidic drugs and warfarin as a single acidic drug

To evaluate how $\bar{n}_{CheqPoint}$ changes with sample weight, $\bar{n}$ at the experimental CheqPoint were calculated with Eq. 2.1.2, and plotted against sample weight. Fig. 2.3.8 shows the $\bar{n}_{CheqPoint}$ vs. weight plots for the five acidic compounds under study. Two clear trends can be observed at first sight, depending on the nature of the compounds that are acids. It should be noticed that the $\bar{n}_{CheqPoint}$ stabilization with the sample weight is done at lower $\bar{n}_{CheqPoint}$ values. Moreover, the more insoluble the analyzed compound, the lower $\bar{n}_{CheqPoint}$ is. This is clearly shown in Fig. 2.3.8 for the paraben series. The four parabens (methyl-, ethyl-, propyl-, and butylparaben) behaved similarly, since $\bar{n}_{CheqPoint}$ decreased as sample weight increased. This trend is strongly marked with ethyl-, propyl-, and butylparaben, and less evident with methylparaben. As pointed out, these compounds belong to a homologous family so that their difference is just the length of the alkyl chain. Due to the hydrophobic character of the alkyl chain, S_o increases as the chain length decreases. In case of the more soluble methylparaben, the curve could not be extended for two reasons; experiments at lower sample weights could not be performed, since the compound did not precipitate, and experiments at higher sample weights implied too much titrant volume in the initial stage of the experiment when the compound is solubilized, so the maximum capacity of the cell was exceeded. Therefore, the curve for this compound is shorter than the one of the other parabens, although the same trend is obvious. For warfarin as a single acid the same behaviour was seen. It means, $\bar{n}_{CheqPoint}$ decreased when sample weight increased. It can be said that, at least in two acidic drugs from different groups, paraben family and single warfarin, the same behaviour has been observed. For all of these acids, an increase in sample weight makes the compound reach faster the maximum concentration of neutral species in solution before precipitation when *pH* is lowered. Thus, precipitation occurs when *pH* is still quite higher than the pK_a of the compounds, i.e., at low

$\bar{n}_{CheqPoint}$. As long as the sample weight decreases, a higher percentage of sample is protonated to form the neutral species before reaching the solubility value, so precipitation occurs at higher $\bar{n}_{CheqPoint}$ values. Fig. 2.3.8 shows a sharp decrease in $\bar{n}_{CheqPoint}$ with increasing the sample weight and, finally, this value is stabilized.

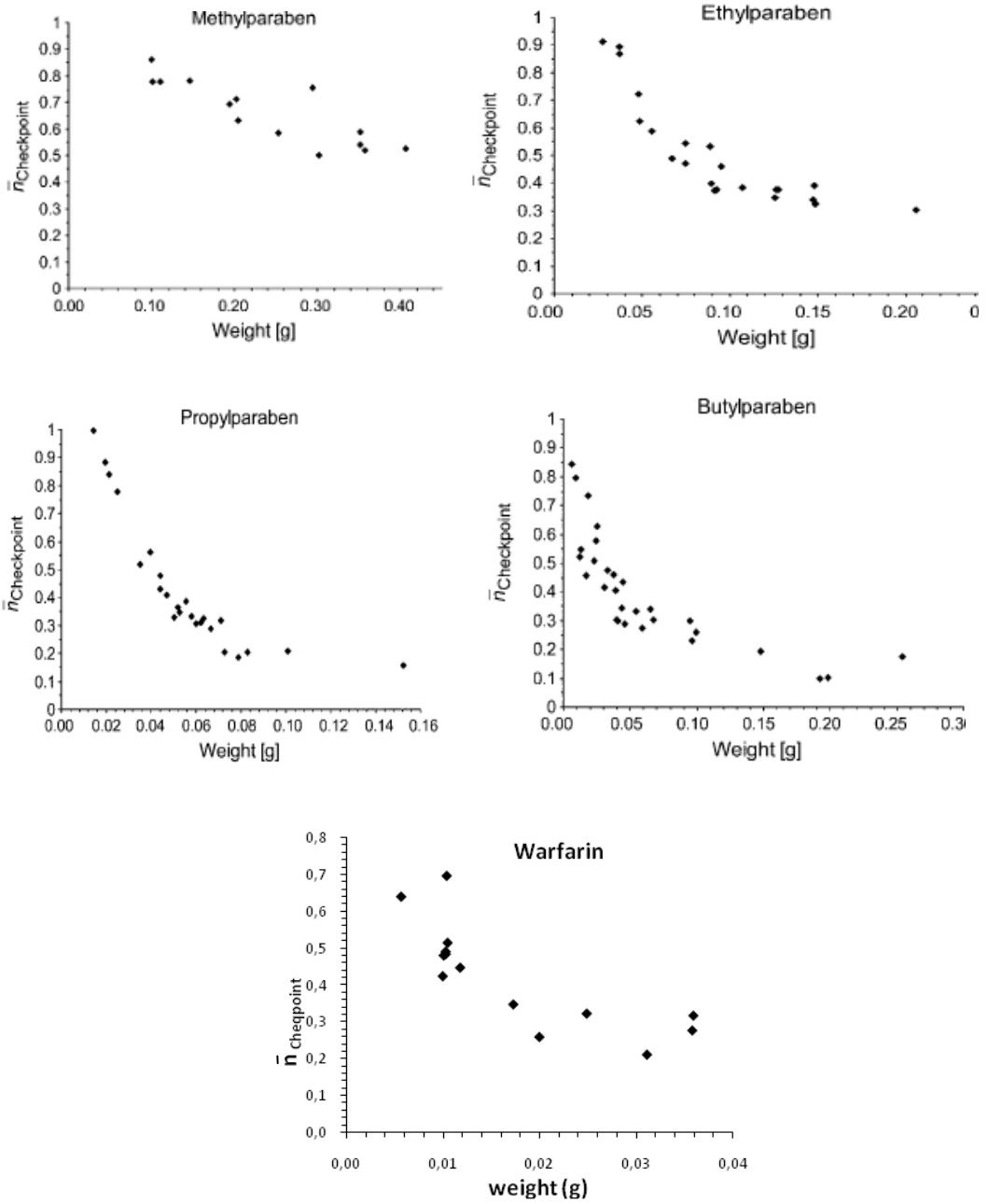


Figure. 2.3.8 $\bar{n}_{CheqPoint}$ vs. sample weight (g) plots of the paraben family and single warfarin

2.3.5.2 4-aminobenzoate family as basic drugs and lidocaine as a single drug

To evaluate how $\bar{n}_{CheqPoint}$ changes with sample weight, $\bar{n}_{CheqPoint}$ values were calculated with Eq. 2.1.3, and plotted against sample weight. Fig. 2.3.9 shows the $\bar{n}_{CheqPoint}$ vs. weight plots for the four basic compounds under study. All the member of the 4-aminobenzoate family behaved similarly, since $\bar{n}_{CheqPoint}$ increased as sample weight increased. Fig. 2.3.9 shows a sharp increase in $\bar{n}_{CheqPoint}$ with increasing the sample weight and, finally, this value is stabilized. This trend is strongly marked with methyl- and ethyl- 4-aminobenzoate and less evident with butyl 4-aminobenzoate. As pointed out before, these compounds belong to a homologous family so that their difference is just the length of the alkyl chain and S_o increases as the chain length decreases. In case of the less soluble butamben, the curve could not be extended for two reasons; experiments at lower sample weights (< 5 mg) could not be performed, since the compound did not precipitate, and complete solubilizing of the compound at higher sample weights (> 13 mg) was impossible. In fact, it was necessary to use ultrasound in order to have complete soluble compound in the solution, but for higher amount of compounds, we needed to put it more time in ultrasound. However, in this way, the temperature increased and that caused the compound to be decomposed. Therefore, the curve for this compound is shorter than the one of the other bases, although the same trend is obvious. For lidocaine also the same behavior was observed, it means, an increase in sample weight implies an increase in the $\bar{n}_{CheqPoint}$ value. This behavior can be explained in all cases according to the corresponding Bjerrum plots. For all studied bases, high sample weights also make the compound precipitate before reaching its pK_a value, thus the percentage of neutral species in solution is very low, and, therefore, high $\bar{n}_{CheqPoint}$ values are obtained. In the same way, when sample weight is decreased, a higher percentage of sample is deprotonated before reaching the solubility value, so that precipitation occurs at lower $\bar{n}_{CheqPoint}$ values. In addition, the studied 4-

aminobenzoate derivatives show low pK_a values for the protonated species. Therefore, the acidic shift of the precipitation curve avoids suitable measurements when the weight of sample is too much high.

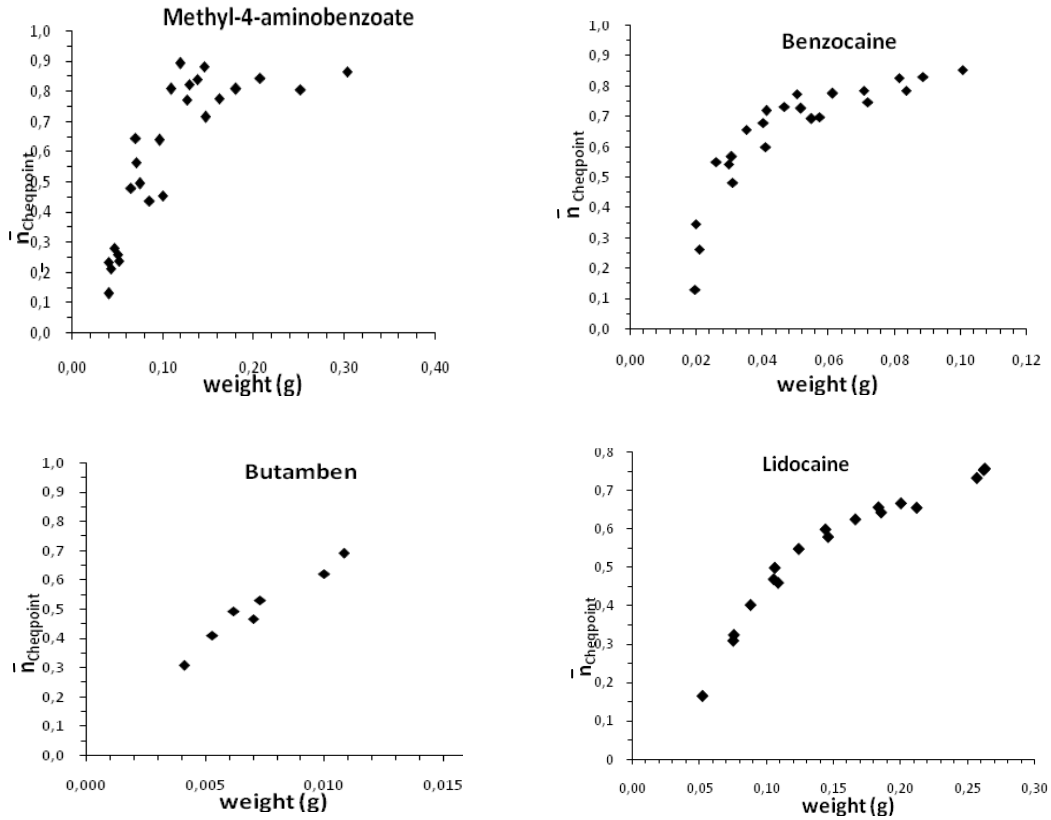


Figure. 2.3.9 $\bar{n}_{CheqPoint}$ vs. Sample weight (g) plots of the 4-aminobenzoate family and single lidocaine

From all these experiments in part 2.3.5, it can be concluded that each compound has a weight range (corresponding to $0.2 < \bar{n}_{CheqPoint} < 0.8$) whose magnitude depends on the S_o of the compound in which the experiments must be performed. Figs. 2.3.3, 2.3.4 and 2.3.5 reveal that the measurements performed outside the recommended $\bar{n}_{CheqPoint}$ range are outliers. These results confirm that working outside the recommended $\bar{n}_{CheqPoint}$ range can lead to wrong S_o values. Figs. 2.3.3, 2.3.4 and 2.3.5 show also there are no significant differences between the obtained

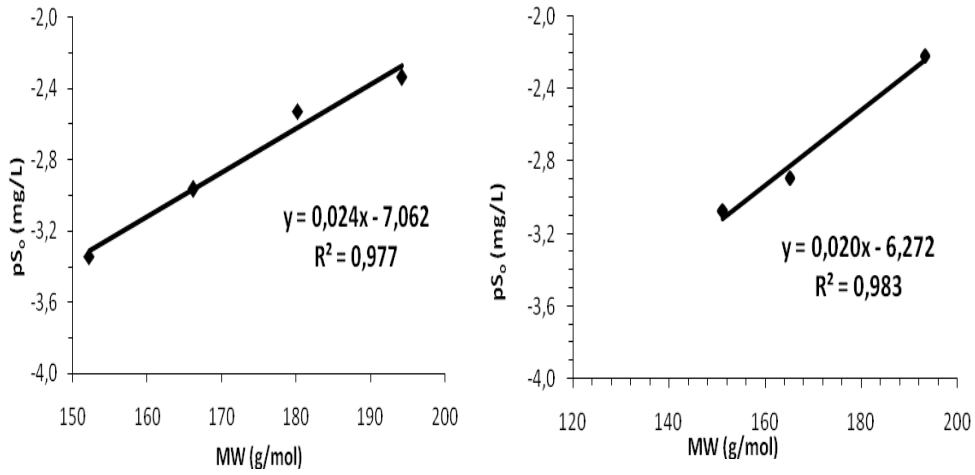
S_o values when sample weight is changed. Therefore, the following weight ranges are recommended for the studied compounds (Table 2.3.3).

Table 2.3.3 Weight range of studied compounds

Compound	Weight range (g)
Methylparaben	0.09 - 0.4
Ethylparaben	0.05 – 0.2
Propylparaben	0.025 – 0.075
Butylparaben	0.02 – 0.1
Warfarin	0.006-0.036
Methyl 4-aminobenzoate	0.06-0.25
Benzocaine	0.02-0.05
Butamben	0.005-0.01
Lidocaine	0.06 – 0.25

However, it is important to note that, according to the exponential shape of curves shown in Figs. 2.3.8 and 2.3.9, a small reduction in sample weight will make the $\bar{n}_{CheqPoint}$ value increase (for acids) or decrease (for bases) very rapidly, so that users must not work beyond the limit of desirable $\bar{n}_{CheqPoint}$ values.

As both parabens and 4-aminobenzoate family seperately belong to a homologous series, it is quite likely that their S_o values are related to their molecular weight (MW). This is why S_o in its logarithmic form (pS) has been represented vs. the MW of each paraben and for each 4-aminobenzoate (Fig. 2.3.10). A straight line is obtained as expected for both series.



(A) **(B)**

Figure. 2.3.10: pS$_o$ (mg/L) vs. Molecular weight (g/mol) plots for (A) parabens & (B) 4-aminobenzoate families

2.3.6 Biopharmaceutical classification of studied compounds

As explained in the General Introduction, the Biopharmaceutical Classification System of the drugs (BCS) depends on two molecular properties, the solubility and the permeability. When permeability is not available, it can be successfully replaced by the lipophilicity, measured as the distribution coefficient between 1-octanol and water (log$P_{o/w}$). The intrinsic solubility for nine different compounds has been determined in this work and literature shows their log$P_{o/w}$ values, which are given in Table 2.3.4 [20]. Then, parabens, warfarin, 4-aminobenzoate derivatives and lidocaine have been classified according to BCS. As shown in Fig. 2.3.11 the single acid, warfarin, is in Class II whereas the single base, lidocaine, is in Class I as already reported [21]. All studied compounds belonging to the two studied families are classified in Class I. So, these compounds can be considered of high solubility and high permeability and, consequently, a high bioavailability can be expected for these substances.

Table 2.3.4 log$P_{o/w}$ and log S_o svalues for studied compounds

Compound	MlogP(BioLoom)	log S_o
Warfarin	2.70	-4.75
MethylParaben	1.96	-1.84
EthylParaben	2.47	-2.26
Propylparaben	3.04	-2.73
ButhylParaben	3.57	-2.95
Lidocaine	2.21	2.86
Methyl-4-aminobenzoate	1.35	-2.11
Benzocine	1.86	-2.33
Butamben	2.87	-3.07

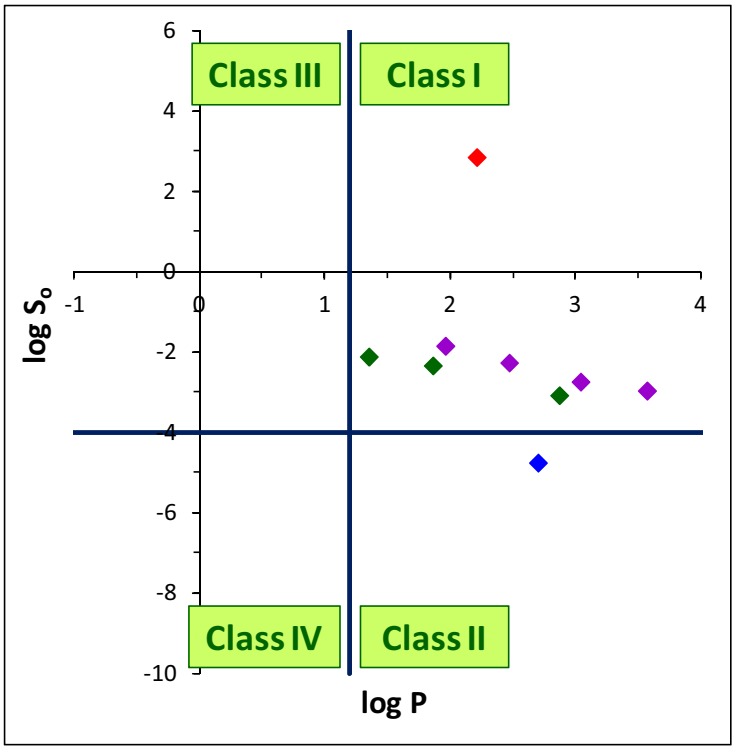


Figure. 2.3.11 Biopharmaceutical classification of studied compounds

Warfarin (◆), Lidocaine (◆), Parabens (◆), 4-aminobenzoates (◆)

In short, the Chasing Equilibrium method offers an alternative to the classical procedures to measure the solubility of compounds with acid-base properties. The method is fast, and accurate results can be easily obtained. The study of the experimental conditions to determine the solubility of several compounds, including both acidic and basic substances, has allowed the establishment of sample weight ranges to get accurate solubility values. Thus, it has been demonstrated that not any sample weight is adequate to obtain reliable results, but only a limited range of weights that depends on the nature and solubility of the compounds.

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