Methods for measurement of solubility and dissolution rate of sparingly soluble drugs

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Abstract

Title:	Methods for measurement of solubility and dissolution rate of sparingly soluble drugs
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Key words:	Rotating disc, dissolution, pharmacopeial method, intrinsic dissolution
Purpose:	The purpose of this paper is to give a wide introduction to dissolution in the Pharmacopeial area and make a pre-study of the rotating disc. The focus will be on determining crucial factors that might affect the method and then make further analysis of the most important factor. Securing the reliability also means investigating the repeatability of the method.
Methodology:	Rotating disc experiments
Empirical foundation:	Interviews, course literature, news articles
Conclusion:	Does the flow conditions influence the measurements? The method used to take samples, the probe, and a relative long sampling time in a moving medium results in a measurement in “volume”. This “volume measurement” reduces the difference in concentration measured. There is a measurble difference, but not big enough to say there is a significant difference.

Summary

The drug discovery process has changed dramatically during the last decade. The development of technologies such as combinatorial chemistry has introduced methods to synthesize massive numbers of new diverse compounds. This, combined with High-Throughput Screening techniques HTS, makes it possible in vast numbers to examine the potency *in vitro* of new compounds. This kind of approach is highly successful in the discovery of new structures with superb *in vitro* potency, but ignores issues of absorption and bioavailability.

The dissolution rate and the release rate of a drug are essential to know for drug characterization. Poor aqueous solubility is likely to give rise to increased formulation difficulties during clinical development. Thus it is of interest to accurately measure solubility of sparingly soluble compounds. In order to measure the dissolution an instrument called rotating disc is used. The big benefit with the rotating disc method is that it uses well defined dissolution process and easy to use equipment

The purpose of this paper is therefore to give a wide introduction to dissolution in the Pharmacopeial area and a make a pre-study of the rotating disc. The focus will be on determining crucial factors that might affect the method.

There is no substantial analysis performed on the Shear distributions for USP 25, the rotating disc. The similarities to USP Apparatus II (same vessel as the rotating disk but a paddle instead of the disk), suggests that uneven shear distributions might be present. Thus it is important to examine the flow conditions.

The concentration has been measured and turbulent conditions have been introduced and second measurements have been taken. There is a difference in the measurements in concentration but not big enough to have any significance. The disturbance could be the result of other factors such as human influence, the tablet or the probe and the measuring device. The concentration in the lower part of the container which gives a higher concentration than the standard run indicates that we have an uneven shear distribution. But the difference is also too small to rule out other factors.

One major factor that affects the results is the method used to take samples, the probe and the relative long sampling time. This sampling in a moving medium results in a measurement in a volume. This volume measurement reduces the difference in concentration variations measured, because an average of the measurement is used.

The conclusion is that there probably are uneven sheer distributions that lead to different concentrations in the vessel. It is very important to use the same sampling point and the same measuring device and sampling times if you want to accurately compare measurements of different compounds.

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1 Introduction

The dissolution rate and the release rate of a drug are essential to know for drug characterization. In order to measure the dissolution an instrument called rotating disc is used. It is of importance to investigate the rotating disc method in order to secure a robust way of dissolution measurements. The big benefit with the rotating disc method is that it uses well defined dissolution process and easy to use equipment which are two important factors to achieve good reproducibility.

The purpose of this paper is therefore to give a wide introduction to dissolution in the Pharmacopeial area and a make a pre-study of the rotating disc. The focus will be on determining crucial factors that might affect the method and then make further analysis of the most important factor. Securing the reliability also means investigating the reproducibility of the method.

2 Background

The drug discovery process has changed dramatically during the last decade.[1] The development of technologies such as combinatorial chemistry has introduced methods to synthesize massive numbers of new diverse compounds. This, combined with High-Throughput Screening techniques HTS, makes it possible in vast numbers to examine the potency *in vitro* of new compounds. This kind of approach is highly successful in the discovery of new structures with superb *in vitro* potency, but ignores issues of absorption and bioavailability.[2] The physical properties of the lead compounds attained by HTS do not necessary keep the potency in the in vivo situation. They reflect the properties of the screening library, usually utilizing enzyme and receptor binding sites.[3] The trend to optimize structure solely on binding site properties generally leads to compounds with high molecular mass and very lipophilic and thus poorly soluble.[4] The apparent “solubility” given by the HTS screen is determined in dimethyl sulfoxide (DMSO) often with surfactants present to improve solubility for the most insoluble compounds. This “solubility” does not reflect the solubility in aqueous solvent under thermodynamic equilibrium conditions. Aqueous solubility is among the first physicochemical parameter measured during the preformulation stage of drug development. Solubility dictates many of the subsequent events and approaches in the formulation development, such as formulations used in early animal bioavailability and toxicity studies. Later the rate of dissolution and stability of the dosage form are determined. Poor aqueous solubility is likely to give rise to increased formulation difficulties during clinical development. Thus it is of interest to accurately measure solubility of sparingly soluble compounds.

3 Dissolution

Dissolution is the process by which a solid substance goes into solution and may be regarded as being composed of two consecutive stages. First an interfacial reaction between solid and solvent breaks up the solid crystal for crystalline substances and opens the amorphous

lattice for amorphous substances. This creates cavities in the solvent, a so called phase change, molecules of solid become molecules of solute.
Secondly the solute molecules are transported away from the interface through a boundary layer by means of diffusion or convection. The boundary layer is close to a wetted surface and is static or very slow moving because of friction and attraction forces, mass transfer here is slow. Thus a concentration gradient arises with a decreasing profile from the saturated solution, C_S in direct contact with the solid to the concentration C of the bulk.

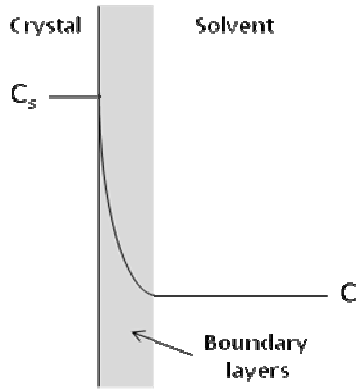

Exhibit 1. Diagram of boundary layers and concentration change surrounding a dissolving particle.

Overall rate of dissolution will depend on the slowest step, the first interfacial reaction is approximately instantaneous, rate determines step is the diffusion through the boundary layer. Fick's law of diffusion states that the change in concentration is directly proportional to the concentration difference over the diffusion layer.

$$J = D \cdot \frac{\partial c}{\partial x}$$

Noyes-Whitney equation describes the dissolution from a single spherical particle, it's a qualitative theory of diffusion kinetics for a heterogeneous reactions and is derived from Fick's law.

$$J = D\frac{(c_S - c)}{\delta}S$$

Nernst & Brunner developed the so-called 'stagnant film theory', a combination of Noyes-Whitney equation and Fick's law of diffusion.

$$J = \frac{dw}{dt} \cdot \frac{1}{A}$$

$$dw \Big/ dt = \frac{DA}{h}(C_S - C); \delta = h$$

where dw/dt is the dissolution rate, D is the diffusion constant, C_S and C are the solubility and bulk concentration respectively, A is the surface area and h is the diffusion layer thickness.

3.1 Intrinsic rate of dissolution

Dissolution is dependent on many factors, both intrinsic and extrinsic. The definition of intrinsic dissolution rate, IDR is the dissolution rate when extrinsic factors are held constant for a pure substance. IDR is influenced by Intrinsic factors, especially particle-size distribution and by extrinsic factors such as hydrodynamics and test conditions. Intrinsic factors are defined by the solid state properties of the pure substance, such as;

- Crystal habit
- Crystallinity
- Amorphism
- Polymorphism
- Pseudo-polymorphism
- Particle size and surface area

The above are predetermined factors which are different for each substance. Extrinsic factors, test conditions, which can be applied to different compounds and show similar trends, need to be addressed further. Because changing them also produces different results.

IDR is the rate of dissolution of pure substances when extrinsic factors as the following are kept constant;

- Agitation
- Surface area of tablet or sample
- Temperature
- pH
- Buffer strength
- Viscosity of the dissolution medium
- Ionic strength of the dissolution medium

IDR is the rate of mass transfer per area of dissolving surface and should be independent of boundary layer thickness and volume of solvent, assuming sink conditions $(C < C_S/10)$. Usually expressed in terms of mg per minute per cm^2.

The Flow Though Cell is accepted in the pharmaceutical industry as a dissolution system when determine dissolution rates. Because there is an established model for dissolution of linear flow past stationary objects. The Flow Though Cell also has the ability to run multiple experiments simultaneously.

4 The Rotating disc

Veniamin Grigorievich Levich studied physics and graduated at the University of Kharkov in 1937 at the age of twenty. At 26 years old he published his first paper giving theoretical consideration of electrical current passing through electrolyte solutions. This was his start as a pioneer for hydrodynamic electrochemistry.[5] He is most famous for the Levich equation, describing the limited mass transfer conditions at a rotating disk. Exhibit 2 show schematic picture of a rotating disk used for dissolution measurements.

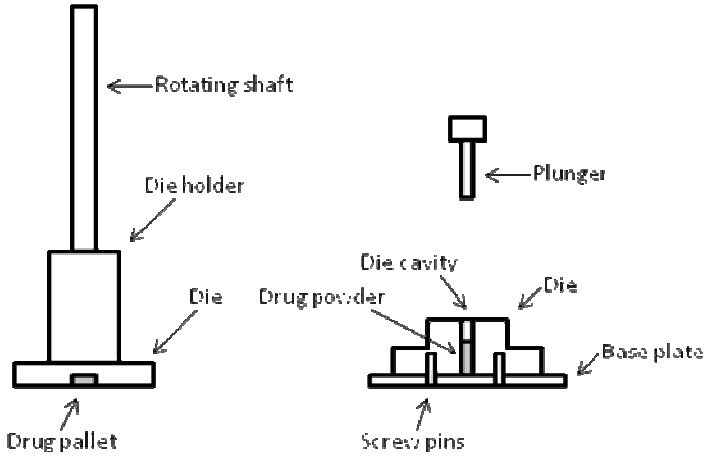

Exhibit 2. USP woods apparatus from VanKel Industries, Inc

The flow generated by a rotating disc can be described by that of a fluid being discharged radially outward from the centre of the disc. A thin layer immediately adjacent to the disc surface acquires a rotating motion. The angular velocity increases as the surface of the disc is approached. The fluid also acquires a radial velocity due to the centrifugal force. There will also be an axial fluid motion toward the disc to preserve continuity.

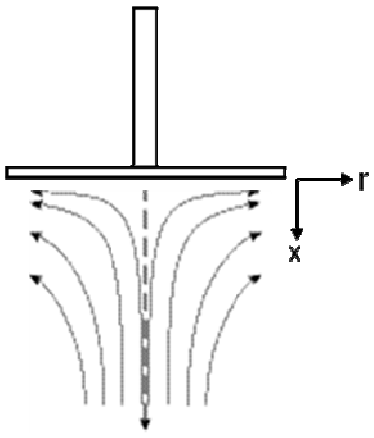

Exhibit 3. Schematic presentation of flow pattern at a rotating disc

The momentum boundary layer formed in connection with friction at the surface of the disc and the diffusion layer formed in connection with diffusional process are both initiated at

the centre of the disc. Exact solutions to the Navier-Stokes equations can be obtained for laminar flow as shown by Levich.

$$J = 0.62 D^{2/3} \nu^{-1/6} \omega^{1/2} C_S$$

Equivalent with

$$\frac{k_C d}{D} = 0.62 \,\mathrm{Re}^{1/2} \, Sc^{1/3}$$

where ω is the rotational speed in radians per second, d is the diameter of the disc, D is the diffusion coefficient, ν is the kinematic viscosity, Re is Reynolds number, Sc is Schmidt number, J is the flux, C_s is the concentration at the surface of the tablet and k_C the mass-transfer coefficient is the same everywhere over the surface of the disk. This model assumes equilibrium at the interface of solid and liquid, and the liquid at the interface is the same as the liquid in bulk dissolution and is only valid under laminar flow conditions. Levich reports that the critical Re lies in the range $1 \cdot 10^4 \leftrightarrow 10^5$.

4.1 Dissolution rate

The rate of dissolution, G can be viewed as the sum of two processes. Namely the true dissolution rate, k_1 and the "rate of re-entry" from aqueous to solid phase, k_2 and k_3 a proportionality factor. For laminar flow an expression for G mg/(cm^2 s) is

$$G = k_1 - k_2 \left(c_0 + k_3 \frac{Ga}{R\omega} \right)$$

For large values of R, disk centre position from rotational axis and large values of ω, the rotational speed in radians per second and a the diameter of the disc, G will approach the intrinsic rate of dissolution k_1. Experimentally k_1 is obtained by measuring $1/G$ as a function of $1/\omega$ and extrapolating to $\left(R\sqrt{\omega}\right)^{-1} = 0$. This gives straight lines with intercepts equal $1/(k_1\text{-}k_2c_0)$. The true k_1 can be found when the concentration gradient governing the diffusion flow is so large that the Fickian flow no longer influences the rate-determining step.[6]

5 Solubility

Solubility is defined as the amount of substance that passes into solution to achieve a saturated solution at constant temperature and pressure. Solubility are expressed in terms of maximum volume or mass of the solute that dissolve in a given volume or mass of a solvent. Pharmacopoeias give solubility's in terms of the number of parts by volume of solvent required to dissolve one part by weight of a solid, or one part by volume of a liquid.

5.1 Intrinsic solubility

In the pharmaceutical industry, the *intrinsic solubility* is measured to indicate solubility. *Intrinsic solubility* corresponds to the equilibrium solubility at thermodynamic equlibrium of the pure uncharged compound/drug. The term intrinsic refers to the fact that the species in solution is the same as that of the solid form. Measurements are taken at two temperatures, first at $4^{\circ}C$ to ensure physical and chemical stability for short-term storage. A minimum in aqueous solubility can be observed at $4^{\circ}C$ because of a maximum of water density. The second temperature of interest is $37^{\circ}C$, to support biopharmaceutical evaluation.

The hypothetical compound/drug/chemical AB illustrates solubility:

$$AB_{(solid)} \xleftrightarrow{K_0} AB_{(so\ln)} \xleftrightarrow{K_0^{'}} A^{+} + B^{-}$$

$AB_{(solid)}$ refers to the chemical in the solid form, $AB_{(soln)}$ to the chemical in solution in an undissociated form and A^{+} and B^{-} to ions of the dissociated solute in solution. Salts from strong acids (Cl^{-}, SO_4^{2-}, NO_3^{-}) are not protonized, they are neutral. An exception is aluminium salts of strong acids, they are basic. Salts from weak acids can be pronized. The equilibrium solubility relationship for a non-electrolyte where the equilibrium constant, K_o, is also known as S_o, the *intrinsic solubility*:

$$K_0 = \frac{(AB)_{so\ln}}{(AB)_{solid}} = S_0$$

The total solubility observed represents the sum of the equilibrium concentrations of all species present. For a non-electrolyte, the total solubility is the intrinsic solubility.

Non-electrolytes, and strong electrolytes where neither A nor B is acidic or basic, generally do not display solubility characteristics that are directly dependent upon pH. But most drugs are weak bases and around 20% are weak acids and only 5% are non-ionic.[7] The Henderson-Hasselbalch equations are used to find the intrinsic solubility for weak bases and acids.

$$pH = pK_a + \log_{10}([B]/[BH^{+}])$$

$$pH = pK_a + \log_{10}([A^{-}]/[HA])$$

A saturated aqueous solution of the salt of a weak base has a concentration equal to the intrinsic solubility of the salt, and it will be relatively acidic (it will have a pH usually more that 2 units below the pK_a of the weak base).
The intrinsic solubility of the weak base is the lowest observed solubility, and it will be observed when pH is more than 2 units above the pK_a.

A saturated aqueous solution of the salt of a weak acid has a concentration equal to the intrinsic solubility of the salt, and it will be relatively basic (it will have a pH usually more than 2 units above the pK_a of the weak acid).
The intrinsic solubility of the weak acid is the lowest observed solubility, and it will be observed when pH is more than 2 units below the pK_a.

5.2 Measuring solubility

In pharmaceutical practice, one important task is to measure the solubility of solids in liquids. The following precautions serve as guidelines when running solubility tests:

- The solvent and the solute must be pure.
- The sample is removed for analysis after confirmation of saturation.
- Sample separation from saturated solution with un-dissolved solute must be reliable and satisfactory.
- The method used to analyse the solution must be reliable and reproducible.
- Temperature must be adequately controlled.

Traditionally, the equilibrium solubility at a given pH and temperature is determined by the shake flask method. According to this method the compound is added in surplus to a certain medium and shaken at a predetermined time, usually 24h or longer. The saturation is confirmed by observation of the presence of un-dissolved material. Saturation can also be reached if the solvent and excess solute is heated and then allowed to cool to the given temperature. Some solutions can hold a certain amount of excess solute in the solvent, commonly called a supersaturated solution. This often occurs when the saturated solution is cooled slowly. Super saturation for salts can be avoided by slow cooling and continuous shaking of the sample during cool down.

After filtration of the slurry a sample for analysis can be taken. Both filtration and analysis should be performed under the same temperature as the solubility determination and under conditions to minimize loss of volatile components. Often the sample is diluted to prevent crystallization. The amount of solute contained in the sample is determined by an appropriate method affected by the nature of the solute/solvent and by the concentration. Common methods are;

- Ultraviolet /visible spectroscopy
- Chromatographic methods
- Gravimetric/volumetric

The shake-flask method is the most accurate method to determine solubility but it is time consuming. Due to the growing need to determine solubility faster new devices[8,9] and automated methods[10,11] have been developed.

1. **Miniaturized shake-flask method** can be used for almost all compounds. Its precision and throughput was proven greater the potentiometric method. Up to 20 compounds per week can be studied with one set-up. The miniaturized shake-flask method needs more drug though to ensure an equilibrium between solid and dissolved drug.[12]

2. **Semiautomated potentiometric acid/base titrations** method is a very economical method and is able to create a pH/solubility profile with one single determination. Only 100 µg of poorly soluble compound is needed. The time required for a potentiometric titration vary depending on the compound, whereas the time consumption with a shake-flask method is fixed but overall it requires more time. However, this method is limited to ionizable compounds.[13]

3. **A computational screening model** is used for the prediction of intrisic solubility, which is based on lipophilicity and molecular surface areas.[14]

4. Another devise, simply called **miniature device**, was developed for measuring aqueous and non-aqueous equilibrium solubility during drug discovery. With only ≈ 1mg of compound, it was possible to determine the entire pH-solubility profile. This devise even got similar solubility values compared to other conventional shake-flask methods.[15]

5.3 Solubility definitions

The descriptive terms for the approximate solubility's of Pharmacopeial and National Formulary substances given by United States Pharmacopeia, USP 23 is presented below.

Table 1. Solubility terms given by USP 23

Descriptive Term	Parts of Solvent Required for 1 part of Solute	g/L in water	M=400 mol/L in water	M=40000 mol/L in water
Very soluble	≤1	≥1000	≥2,5	≥0,025
Freely soluble	1 to 10	1000 to 100	2,5 to 0,25	0,025 to 0,0025
Soluble	10 to 30	100 to 33	0,25 to 0,08	0,0025 to 0,0008
Sparingly soluble	30 to 100	33 to 10	0,08 to 0,025	0,0008 to 0,00025
Slightly soluble	100 to 1000	10 to 1	0,025 to 0,0025	0,00025 to 0,0000025
Very slightly soluble	1000 to 10,000	1 to 0,1	0,0025 to 0,00025	0,000025 to 0,0000025
Practically insoluble, or Insoluble	≥10,000	≤0,1	≤0,00025	≤0,0000025

5.3.1 Biopharmaceutics classification system

The BCS[16] is a framework for classifying a drug substance based on its aqueous solubility and intestinal permeability. BCS introduces an *in vitro-in vivo* correlation and is the first step for approving a product based on *in vitro* dissolution tests rather than bioequivalence studies in

human subjects. As a result unnecessary human experiments can be avoided thus lowering development costs of new drugs.
Solubility is based on the highest dose strength and is considered highly soluble if soluble in 250 mL or less of aqueous media over the pH range of 1.0-7.5, otherwise considered to be poorly soluble.
Permeability is based indirectly on the measurement of the rate of mass transfer across the human intestinal membrane. Models capable of predicting the extent of intestinal absorption in humans may be used as alternatives. A drug substance is considered highly permeable when the intestinal absorption is determined to be 90% or higher, otherwise considered to be poorly permeable.
Combined with *in vitro* dissolution characteristics of the drug, BCS takes into account all factors that govern the rate and extent of oral drug absorption for immediate release dosage forms. The theoretical considerations suggested by Amidon 1995 describes a simplified model with three dimensionless numbers controlling drug absorption; Dose Number D_0, Dissolution Number D_n and Absorption Number A_n, and a This model gives a basis for determine when and under what conditions *in vitro-in vivo* correlations are to be expected. The definitions are shown below.

$$D_0 = \frac{M_0 / V_0}{C_s}$$

$$D_n = \frac{DC_s}{r_0} \cdot \frac{4\pi r_0^2}{4/3\pi r_0^3 \rho} \cdot t_{res} = \frac{t_{res}}{t_{diss}}$$

$$A_n = \frac{P_{eff}}{R} \cdot t_{res}$$

$$t_{res} = \frac{\pi R^2 L}{Q}$$

$$t_{diss} = \frac{r_0^2 \rho}{3DC_s}$$

Where the dose drug administrated M_0, the initial gastric volume V_0, the saturation solubility C_s, the diffusion coefficient D, the initial particle radius r_0, the density ρ, tube length L, fluid flow rate Q, tube radius R, the mean residence time t_{res}, the time required for a particle of the drug to dissolve t_{diss}, the effective permeability P_{eff}, and the segment radius R.

BCS introduces four classes according to solubility and permeability of the drug. The permeability of the drug is hard to obtain and is often derived from the fraction drug absorbed. For class I drugs *in vitro-in vivo* correlation (IVIVC) may be expected if dissolution rate is slower than gastric emptying rate. For class II drugs an IVIVC is expected if *in vivo* and *in vitro* dissolution rates are similar. Fot class III drugs limited to no IVIVC is expected because the absorption is the rate determine step. Class IV show limited or no IVIVC.

Table 2. In Vitro.in Vivo Correlation (IVIVC) Expectations for Immediate Release Drugs Based on BCS

Class	Solubility	Permeability	IVIV Correlation Expectation
I	High	High	IVIVC if dissolution rate is slower than gastric emptying rate, otherwise limited or no correlation
II	Low	High	IVIVC exected if in vitro dissolution rate is similar to in vivo dissolution rate, unless dose is very high
III	High	Low	Absorption is rate determine and limited or no IVIVC with dissolution rate.
IV	Low	Low	Limited or no IVIVC

6 USP 25

United States Pharmacopeia is an official public standards–setting authority that generate documentary standards and reference standards to be used by regulatory agencies and manufacturers of pharmaceuticals.[17] The number after USP refers to a specific standard, in this case 25 refers to the rotating disc.

Weigh the material to be tested onto a piece of weighing paper. Attach the surface plate to the underside of the die, and secure it with the three screws. Transfer the accurately weighed portion of the material under test into the die cavity. Place the punch into the chamber and compress the powder for 1 minute at a minimum compression pressure necessary to form a non-disintegrating compacted pellet.

Detach the surface plate, and screw the die with punch still in place into the holder. Remove all loose powder from the surface of the die by blowing compressed air or nitrogen. Position the shaft in the spindle so that when the disc is lowered, the exposed surface of the material will be 3.8 cm from the bottom of the vessel. The disk assembly should minimize wobble, and air bubbles should not be allowed to form on the compacted material or die surface as this could alter hydrodynamics.

Measure the amount of test material dissolved until 10% of the sample is dissolved. It is important to notice that the surface of the sample move into the die when material is dissolved. If the surface is allowed to travel to far into the die the hydrodynamic conditions for Levich equations are not fulfilled and the data can not be used accurately. Finally plot the cumulative amount of test specimen dissolved per unit area of the compacted material against time.

6.1 Flow problems in USP Apparatus II

United States Pharmacopeia also provides standards for equipment used in the manufacturing of pharmaceuticals, dietary supplements, and food ingredients to ensure quality. USP Apparatus II describes the solubility method and the equipment used.

Current dissolution instrumentation is a highly variable technique due to incomplete mixing. The geometric parameters of the dissolution vessel and stirring mechanism lead to flow patterns that create different concentration densities.[18] A comprehensive analysis of the

Shear distribution in the USP apparatus 2 show that uneven distribution of hydrodynamic forces is a direct cause of dissolution testing variability.[19] This variability is large enough for type II errors, failures resulting from variability of the testing method rather than a problem with dosage form. It is reasonable to assume that uneven distribution of hydrodynamic forces leads to incomplete mixing and there for uneven distribution of solute within the vessel

6.2 Flow problems in USP 25

There is no substantial analysis performed on the Shear distributions for USP 25. But the similarities to USP Apparatus II; same vessel as the rotating disk and the disk works as a paddle, suggest that uneven shear distributions might be present. USP 25 uses slow rotating speed because the method is dependent of a laminar flow. Slow rotating speeds and laminar flow leads to an uneven distribution of hydrodynamic forces and incomplete mixing. Because the rotating disk is dependent of laminar flow nothing can be done about the possible incomplete mixing. This implicates the importance of how and where in the vessel the measurements are made.

7 Measuring Method

Dissolution apparatus, such as Varian, uses probes that take samples of the solution. The negative factor with this kind of sampling is that the probe needs a fixed mount of time in the vessel depending on the sample size. Different measurement methods need a minimum sample size to work and often extra volume to evacuate the chamber where measuring takes place. The small pipes connecting the probe to the measurement equipment is an additional volume added to the sampling volume.
This means a problem because long sampling times, some times up to 1 minute, will influence the hydrodynamic conditions. A long sampling times results in the measuring point becoming equivalent to a volume. Dissolution continues during the sampling and this might be a problem for highly soluble substances.

7.1 Measuring Point

Standard USP 25 praxis is to measure in the middle between the surface and the end of the paddle/disc. This point is good because the shear forces at the measuring point are low compared to the forces around the disk. The device used for measuring or sampling ought to spend as little time as possible in the vessel to minimize the influence on hydrodynamic conditions. A measuring point that influences the flow at the bottom of the disk as little as possible is desirable. A sample close to the surface makes the least influence on the flow but gilds a lower concentration than average. A sample in the same plane as the rotating disk gives a higher concentration than average and significantly affects the flow.

7.2 Compactation pressure

The pressure needed to keep disk hardness and avoid cracks depends upon the diameter of the die; a larger die requires greater compression force.
Pressure used to compact the powder can induce polymorphic form change, which may result in none representative measurements.[20]
For higher compression forces certain substances express more fragile disks that fragment in the dissolution medium.[21]

7.3 Rotating Speed

Recommended rotating speed for wood's rotating disc is 50rpm. Experiments show a increase in the rate of dissolution of a drug when rotating speeds are increased.[22] But high rotational speeds eventually lead to a turbulent flow which is unwanted. Recommended rotating speeds ought to be used to simplify comparison of dissolution profiles.

8 Test plan

Find a suitable test substance that is commonly used in the pharmaceutical area and has a reasonable solubility in water. Identify the wavelength absorption maximum for the test substance. Optimize probe parameters for best possible measurements. Perform dissolution profile experiment according to USP 25. For the experiments acetylsalicylic acid is used as test substance.

8.1 Test Method

Test material was placed in the 0.8 cm diameter die cavity. The punch was inserted into the cavity and compressed for 1 min. Die removed from base plate and attached to die holder, which was then screwed onto the shaft holder. The shaft was mounted on the stirring drive mechanism of the dissolution apparatus. The disc was lowered into the dissolution vessel containing 900ml evacuated de-ionised water at 37^{o}C. Dissolution was performed at 50rpm and every 15 minutes a probe was used to measure the absorbance.

8.2 Validation of probe

Two types of heads could be attached to the probe. Since we are measuring low absorbance's a longer light path is preferred compared to a shorter one. The large head in a solution of 0.156g/l acetylsalicylic acid in water at 37^{o}C with 5 replicates and every replicate consisting of 4 measurements. After studying the SD for the different sample times, 2 seconds was considered a good choice because longer sample times didn't further increase the SD notably.

Table 3. Absorbance data for the large probe head.

Sample time	Mean (s)	Mean	SD (s)	SD
0.0125	0.6384	0.6411	0.003	0.0029
0.1	0.6407	0.6432	0.0025	0.0016
0.2	0.6409	0.6443	0.0016	0.0019
1	0.6411	0.6451	0.001	0.0012
2	0.643	0.6447	0.0004	0.0006
3	0.6439	0.6445	0.0011	0.0004
4	0.6439	0.6444	0.0005	0.0005
5	0.6431	0.646	0.0005	0.0007

8.3 Calibration curve

Absorbance measurements of acetylsalicylic acid at five know concentrations were recorded. Linier regression was performed on the data and a line was fitted under the condition that it passes through (0,0) the result was $y = 0.9129x$. This relation ship is used in the experiments to correlate Absorbance to Concentration.

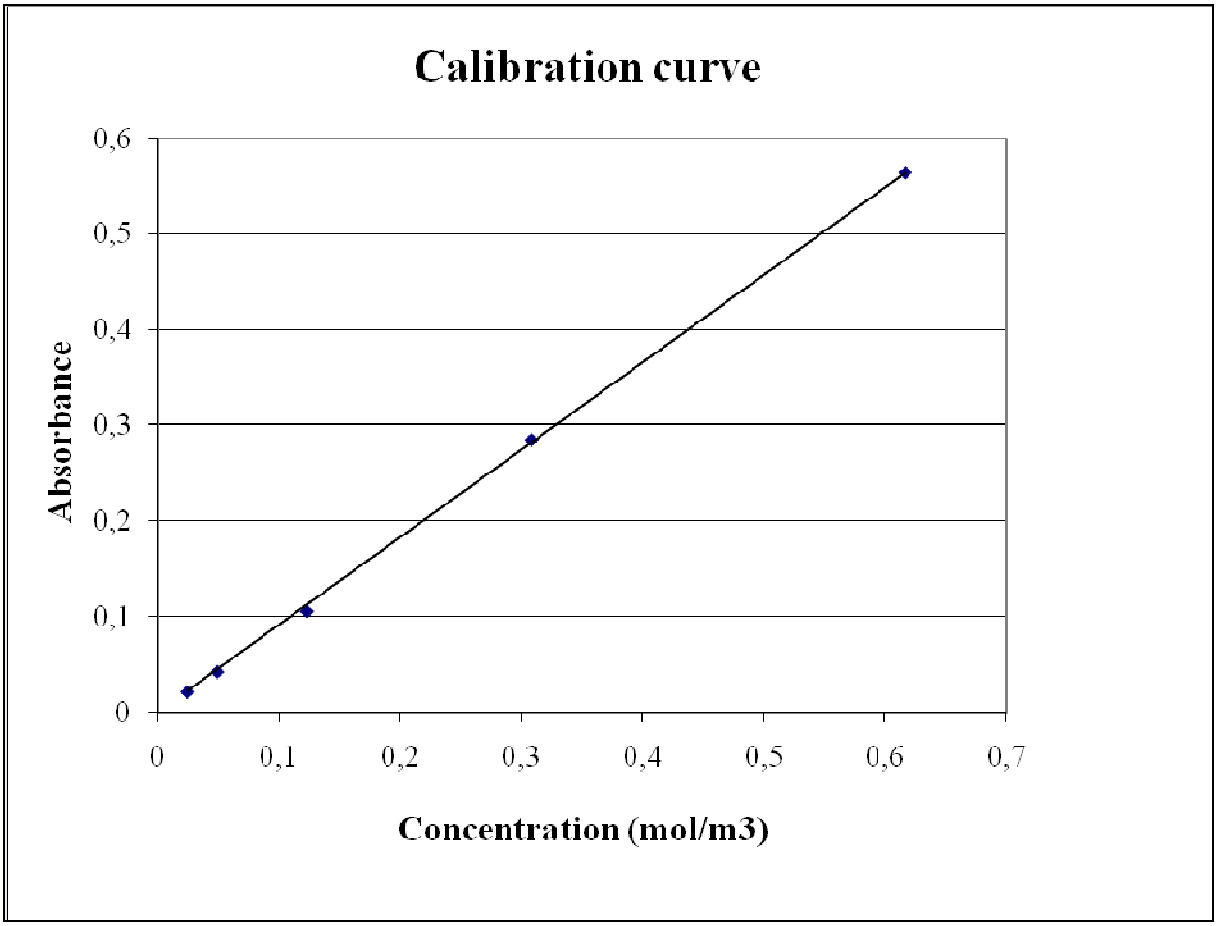

Exhibit 4. Calibration curve with fitted regression line $y = 0.9129x$

8.4 Standard point

Five replicates of concentration measurements of acetylsalicylic acid every 15 minutes for 6 hours. The result is a fitted regression line $y = 0.08226x - 0.01607$. The data displays a linear relation ship to time and none significant outliers can be found. This result is expected from the theoretical background described in chapter 4 of this paper.

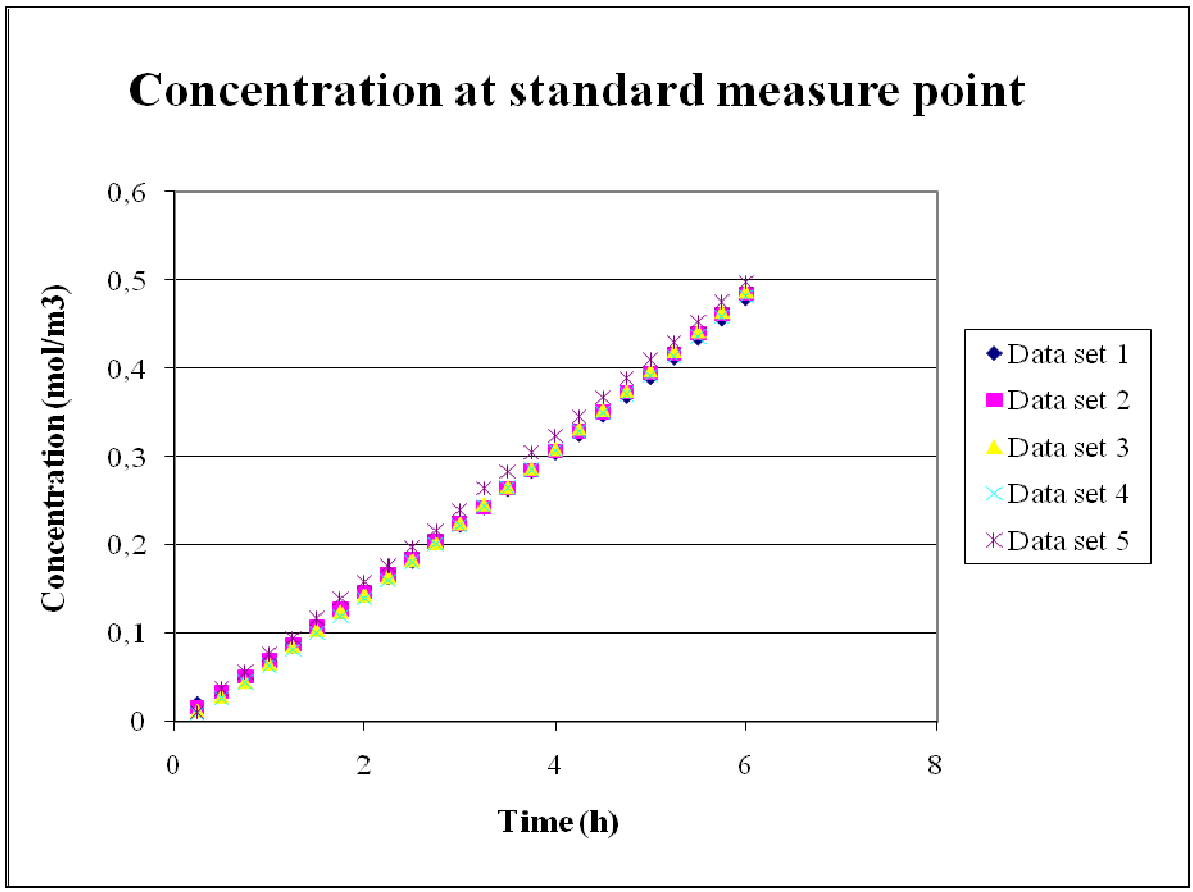

Exhibit 5. Fitted regression line is $y = 0.08226x - 0.01607$

8.5 Flow conditions

The special flow patterns created by the rotating disc under laminar conditions suggest that a highter concentration ought to be found in a plane parallell to the surface of the rotating disc. Here the just released particels flows raially out from the center of the disc. If the concentratin is significant different compared to the standart point then it is safe to say that we have incompleate mixing in the vessel and that the measurments we collect does not represent the true consentration.

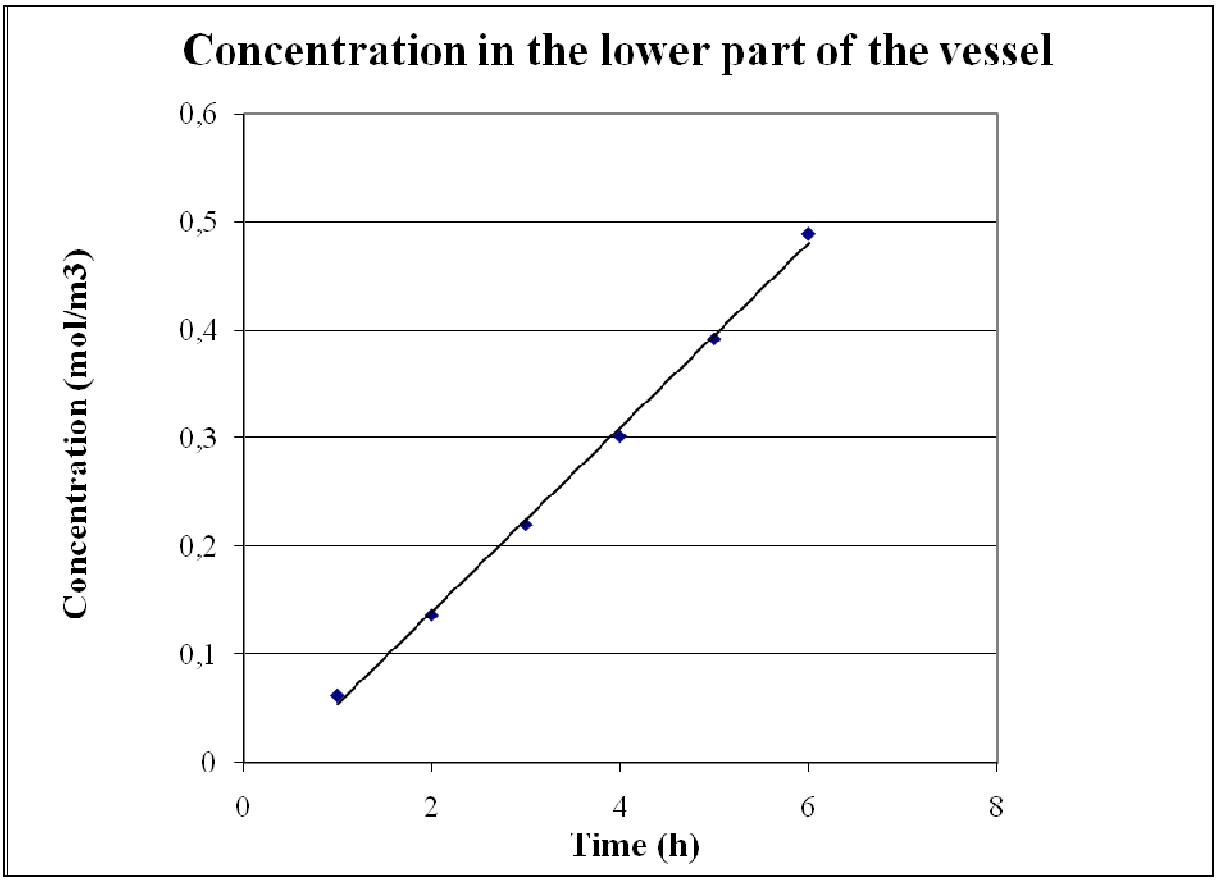

Exhibit 6. Fitted regression line $y = 0.0852x - 0.0310$

The following experiment is performed in individual runs. After a measurement the rotating disc is removed and baffles and a propeller is applied to induce turbulent conditions. A second measurement is taken under these conditions. If the different profiles given by this experiment are significant different, flow conditions do influence the rotating disc method.

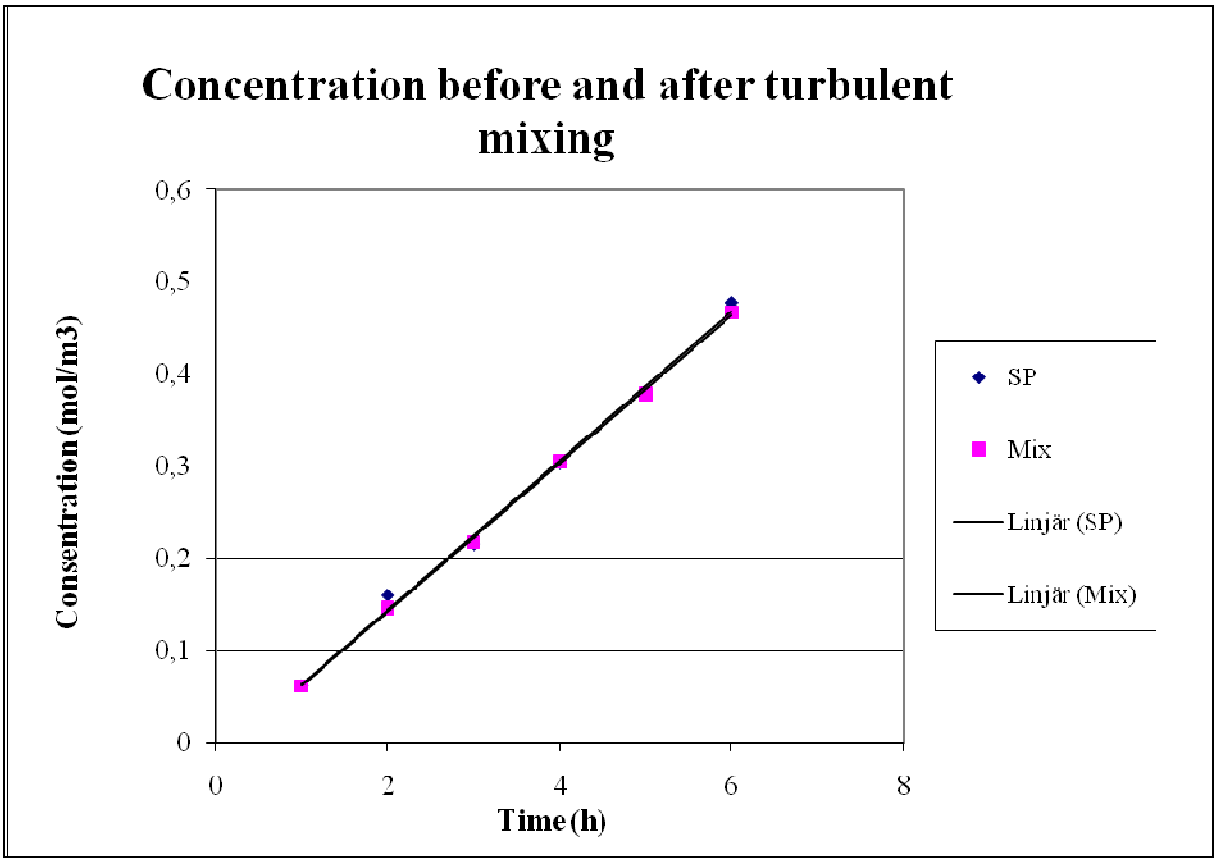

Exhibit 7. Fitted regression line for SP $y = 0.0808x - 0.0164$ and Mix $y = 0.0804x - 0.0185$

8.4 Analysis of test results

Residuals in time sequence are plotted to investigate the errors in the assumption that we have a linear relationship between the concentration and time. By looking at the graph it is obvious that we have a quicker dissolution in the beginning and in the end compared to the middle of the experiment.

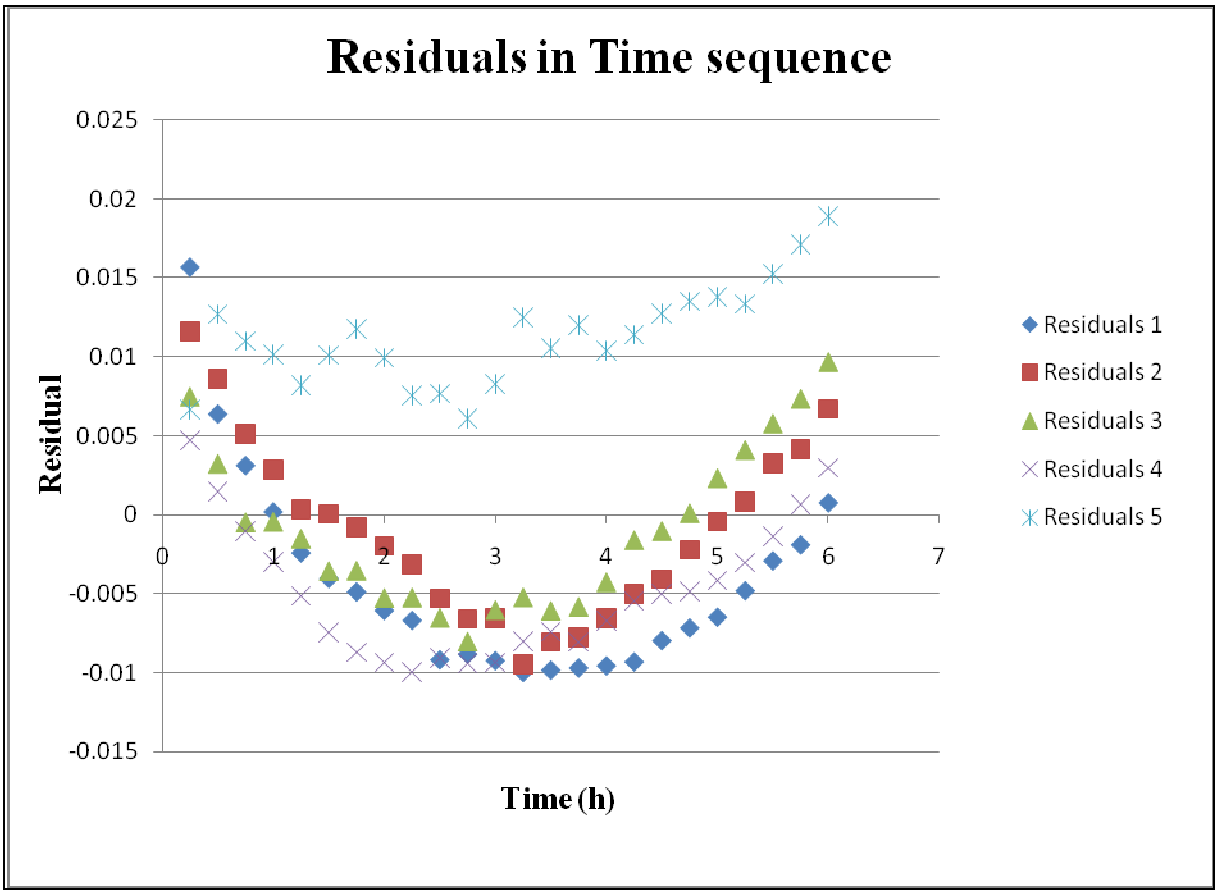

Exhibit 8. Residuals in time sequence show a correlation which imply that the independence assumption of the errors has been violated.

There are possibly both physical and chemical factors which affect the measurement. We can assume that we have a larger driving force early in the experiment because of the concentration difference. In the end of the experiment the pallet has dissolved and the surface has moved inwards in the disc. This change in structure could induce turbulence close to the pallet surface, which increases the dissolution. A linear model in not adequate to describe a profile of acetylsalicylic acid because of the way data is collected. The structure of the residuals suggests that polynomial regression will give a better fit to the data. The scale of the residuals are very small so but enough to indicate that a better fit can be made by a non linear model.

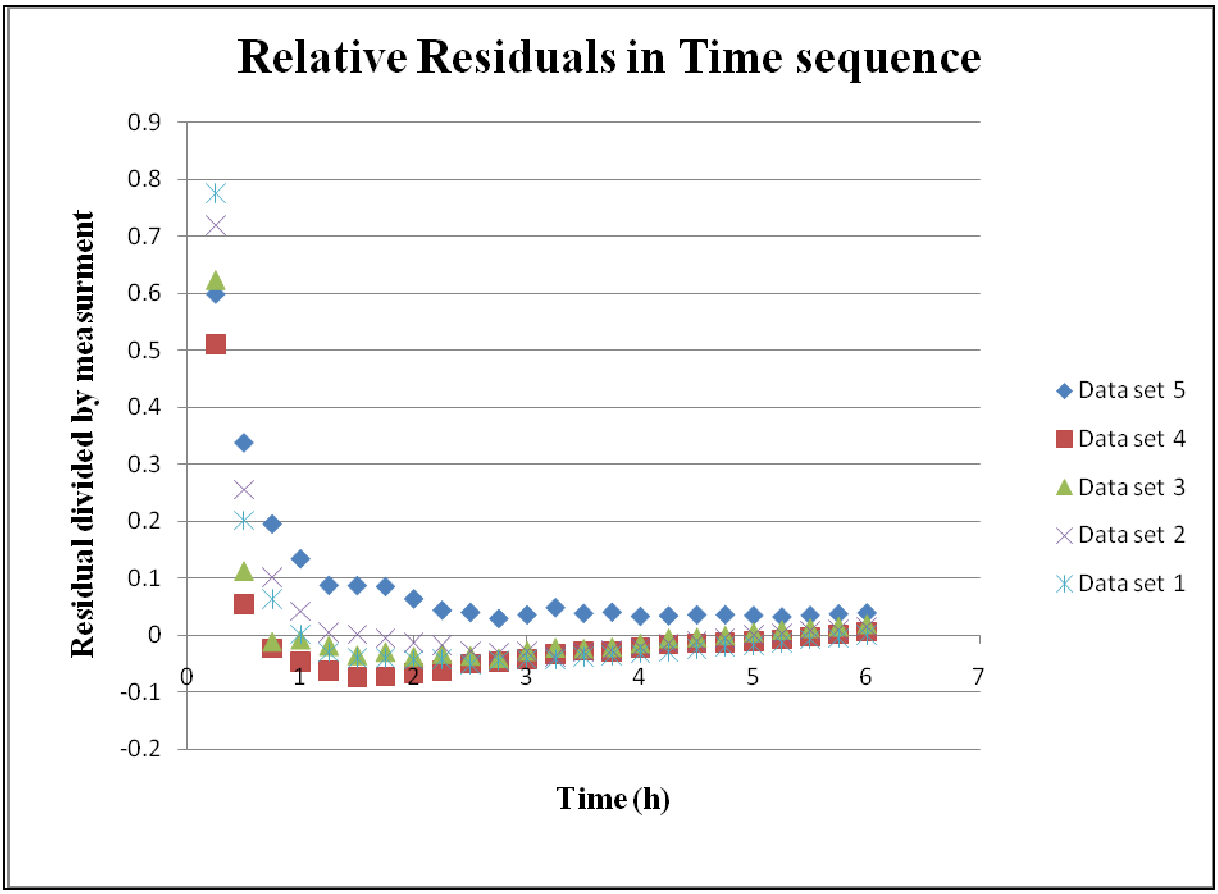

Exhibit 9. Residuals divided by measurement in time sequence show a large deviation from the model the first hour.

Exhibit 9 displays that a large part of the model error occurs during the first hour of the measurements. It is likely that dissolution is slower in the beginning due to bad wetting of the tablet surface. Another influential factor is the flow conditions, when concentrations are low a small disturbance gives a larger effect.

9 Dissolution profile

The amount of drug dissolved per unit area is plotted against time. The slope of the line is the intrinsic dissolution rate, IDR. UPS recommends that earlier time points be used in the calculation of slope. At least five points from the first part of dissolution curve will provide meaningful data. The similarity factor, f_2 are used to evaluate differences/similarities between dissolution profiles.

$$f_2 = 50\log\left\{\left[1+\frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0.5}\cdot 100\right\}$$

Where R_t is the reference data at time point t, T_t is the test data at time point t. n is the number of sampling points. The data are the cumulative percentage dissolved. The value

obtained, f_2 lies in the range 0-100 and values of $f_2 \geq 50$ indicate similarity of two profiles under the assumption of maximum allowable difference of 10%.[23]
There are other methods of comparison especially ANOVA-based approaches which intuitively ought to be good. But they are overly discriminating and tend to answer the question "Are the means of each profile different?" instead of "Are the profiles pharmaceutically indistinguishable?" This is surprising because ANOVA-based approaches are usually a very good tool.[24]

10 Conclusion

The answer to the question if the flow conditions influence the measurements is both yes and no. There is a difference in the measurements in the experiment before and after turbulent mixing, but not big enough to say there is a significant difference. The disturbance could be the result of other factors such as human influence, the tablet or the probe and the measuring device. The concentration in the lower part of the container which gives a higher concentration than the standard run indicates that we have an uneven shear distribution. But the difference is too small to rule out other factors as a part of the influence from the standard run. The method used to take samples, the probe, and a relative long sampling time in a moving medium results in a measurement in a volume. This volume measurement reduces the difference in concentration measured, because an average of the measurement is used.

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