Chapter 1

CORRELATION BETWEEN IN VITRO DISSOLUTION AND BIOAVAILABILITY OF DRUGS

1.1. Dissolution and its correlation with bioavailability of drugs:

For oral solid dosage forms dissolution test is the only in vitro quality control test available till date which can provide an insight to predict in vivo behaviour, bioavailability, the rate at which and the extent to which a drug reaches systemic circulation or site of action from an administered dosage form, of the drug product. Dissolution test serves as a tool to distinguish between 'acceptable' and 'unacceptable', bioequivalent or bioinequivalent, drug products. The value of the dissolution test as a quality control tool is significantly enhanced if an in vitro-in vivo correlation (IVIVC) has been established.1

Drug absorption or drug bioavailability after oral administration of a solid dosage form depends on the release of a drug from its formulation, solubilization or dissolution of the drug under the physiological conditions of the gastrointestinal tract (GI tract) and permeation across the GI tract. As first two of these steps are very critical or rate determining, therefore, in vitro dissolution of a drug becomes important in order to predict accurately in vivo performance of that formulation.

Dissolution conditions, established on the basis of valid correlation of in vitro dissolution with the in vivo behaviour of the formulation, are used to assess the lot-to-lot quality of the drug product, guide development of new formulations, ensure the continuing product quality and performance.

With the fundamental work of Noyes and Whitney (1897) on dissolution and modification of Noyes and Whitney equation by Nernst and Brunner (1904) using Fick's law of diffusion to apply the concept of dissolution to chemical substances led to evolution of the science of dissolution. The dissolution testing became a mandatory requirement for several dosage forms in late 1960s2.

Role of dissolution testing:

The goal of dissolution testing is to assure the pharmaceutical quality of a product in terms of the ability to manufacture the product reproducibly which maintains its release properties through out the shelf life. It should also assure the biopharmaceutical characteristics (e.g. rate and extent of absorption) of the product. Thus, the most desirable aspects of a dissolution test are a) it should assess the ability of a dosage form to release the drug completely and b) it should provide indication of the performance of a dosage form in vivo3.
Dissolution tests are effectively used in –

1. Investigation of drug release mechanism studies, especially for modified release (MR) products
2. Formulation development to reach a predefined target release profile and robust drug release properties with respect to influence of physiological factors (e.g. pH and food)
3. Generating supportive data for interpretation of bioavailability studies
4. Validation of manufacturing process
5. Storage stability studies
6. Batch quality studies
7. Batch quality control
8. As surrogate for bioequivalence studies

Over the last 25 years dissolution testing has emerged as a highly valuable in vitro test to characterize the drug product performance. It plays an important role in development and quality control of drug products – a) It is used to guide development of drug formulation with desirable bioavailability; b) It is used as a batch-to-batch quality control test before the product is released into the market, c) It forms the basis for setting quality control specifications - test and methodology of dissolution, acceptance criteria - in case of oral solid dosage forms to permit the batch release; d) It also helps to identify bioavailability problems and in case of scale-up and post-approval changes it is used to assess the need for further bioequivalence.

However, for the test to be useful, it should be simple, reproducible, and reliable and should be able to discriminate between varying degrees of product performance. When the product performance is examined as a function of time, in terms of multi-point dissolution test or dissolution profile determination rather than single point estimation, the value of dissolution testing is significantly enhanced.

Over the last decade, the dissolution test has moved away from traditional QC test to a surrogate marker for bioequivalence test, thus, enabling to waive the bioavailability studies for certain class of drugs and formulations.

Although dissolution test is the only in vitro QC test which provides insight into bioavailability of a drug product, correlation between dissolution and bioavailability of a drug cannot be taken for granted. It needs to be demonstrated convincingly, because dissolution and bioavailability properties are governed by several factors.
Factors affecting dissolution and bioavailability of the dosage forms:

Since bioavailability of a drug after oral administration is governed by release of the drug from its formulation, solubilization/dissolution of drug under physiological conditions and the permeation of the drug through GI tract, the factors which influence these processes are ultimately responsible for a product's in vivo performance. These factors can be broadly classified as follows:

A. Factors related to physicochemical properties of a drug

These factors govern the dissolution and permeation properties of a drug and are listed below:

a) Lipophilicity
b) Solubility
c) Degree of ionization (pKa)
d) Molecular size and shape
e) Hydrogen-bonding potential
f) Solid phase characteristics - amorphicity and crystallinity (Polymorphism)
g) Coprecipitation and/or complexation
h) Particle characteristics - particle size, particle shape, particle density

B. Factors related to formulation and dissolution testing:

These factors influence the rate of release or solubilization or dissolution of drug from a formulation and can be subdivided as

1. Factors related to drug product formulation:

a) Excipients and additives - diluents, binders, lubricants, granulating agents, disintegrants etc.
b) Particle size (of the drug in granules, tablets etc.)
c) Granulating agents and binders (type, amount, source etc.)
d) Disintegrating agents
e) Lubricants
f) Interfacial tension between drug and dissolution medium
g) Surfactants

2. Factors related to dosage form:

a) Manufacturing procedures
b) Granule size (important in case of harder and slow disintegrating granules)
c) Drug excipient interactions
d) Compression force
e) Deaggregation
f) Storage of dosage form
3. **Factors related to dissolution testing.**
   a) Eccentricity of agitation (stirring) element
   b) Vibration
   c) Agitation intensity
   d) Stirring element alignment
   e) Flow pattern disturbances
   f) Sampling probes, position and filters
   g) Dosage form position
   h) Type of device

4. **Factors related to dissolution test parameters.**
   a) Temperature
   b) Dissolution medium - Dissolved gases (air)
      - Dissolution media composition and pH (surface tension)
      - Viscosity
      - Other factors - Surface tension, interfacial tension, Evaporation of medium, Preparation of medium (Fresh/Old), Volume of dissolution medium.

5. **Miscellaneous factors.**
   a) Adsorption
   b) Sorption
   c) Humidity
   d) Detection errors

**C. Factors affecting in vivo performance of a formulation.**

Once the drug is released from its formulation in the GI environment following factors regulate its absorption into the systemic circulation-

1. **Biological variability (Physiological factors):**
   a) Membrane transport
   b) Gastrointestinal motility (gastric emptying)
   c) Food and other drugs (alteration of dissolution rate, gastric emptying time, pH of stomach fluid, complexing)
   d) Age, weight, activity, disease state

2. **Pharmacokinetic factors:**
   a) Presystemic metabolism (GI and liver metabolism (first pass effect))
   b) Instability of the drug to gastrointestinal contents and enzymes (Chemical instability)
   c) Complexation
   d) Absorption
   e) Distribution and elimination
So, establishment of proper dissolution standards reflecting in vivo performance of a drug is important. Recently, Horter and Dressman have reviewed, extensively, physicochemical properties of drugs which influence their dissolution in the gastrointestinal tract.

Sometimes, the in vitro dissolution test is found to be more sensitive and discriminating than the in vivo test. More discriminative dissolution test is preferred from the quality assurance point of view, because it will throw light on possible correlations or changes in the product quality before affecting the in vivo performance.

Modified release (MR) or Sustained release (SR) or Extended release (ER) formulations are preferred dosage forms for better patient compliance and the dissolution testing for such formulations becomes critical to ensure proper release of the drug. For example, monograph for theophylline ER capsules in USP XXIII and its supplement 9, indicate nine different dissolution test conditions for twice-a-day preparations and a separate drug release test for once-a-day preparations with different dissolution limits and insists that the label should claim which type of theophylline release test the present formulation passes.

Further, one has to recognize that testing of each batch for bioavailability on humans is impractical, expensive, unethical and time consuming. Therefore, in order to achieve the batch-to-batch bioequivalence, there is a need to correlate the data obtained from bioavailability and bioequivalence studies with in vitro quality control procedures (mainly dissolution test).

The concept of in vitro-in vivo correlation, IVIVC, for ER dosage forms in particular, has been extensively discussed. It continues to be a long sought goal to predict, accurately and precisely, expected bioavailability characteristics for an ER product from its in vitro dissolution profile characteristics. This is because the dissolution behaviour of a drug from ER or MR dosage forms in the GI tract is the controlling factor for its absorption. It assumes great importance especially for such formulations which contain drugs having narrow therapeutic window, such as theophylline, diltiazem, carbamazepine, lithium carbonate, nifedipine.

Thus, it is of utmost importance to design a proper in vitro dissolution rate test, under well defined test conditions and correlate the data with the bioavailability data obtained after carrying out the in vivo absorption and bioavailability studies. Once correlated well with the in vivo bioavailability data, such a standardized dissolution test will help predict in vivo performance of a formulation and therefore, can serve as a validated quality control check to assure batch-to-batch reproducibility of formulations with respect to their physiological performance. Such a validated dissolution test can minimize the use of extensive, expensive and time consuming bioequivalence studies involving humans as the subjects.
dissolution test should also indicate the dissolution stability and hence continued physiological performance of the formulation.

Dissolution test requirements: General considerations

Generally compendial monographs incorporating dissolution test and specifications for specific products provide a means to monitor batch-to-batch variability of these products. The two major aspects which decide the outcome of such a dissolution test are:

a) The type of dissolution test apparatus, and
b) the dissolution test conditions employed — e.g., medium, composition, volume, speed of rotation, temperature, etc.

USP has enlisted different types of dissolution test apparatus along with their specifications testing of a variety of formulations ranging from tablets to Transdermal drug delivery systems, which are as follows (Table 1.1).

**Table 1.1**

<table>
<thead>
<tr>
<th>Apparatus</th>
<th>Description</th>
<th>Formulation type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rotating basket</td>
<td>Floating dosage form, Capsules</td>
</tr>
<tr>
<td>2</td>
<td>Rotating paddle</td>
<td>Tablets</td>
</tr>
<tr>
<td>3</td>
<td>Reciprocating cylinder</td>
<td>Bead type MR dosage forms</td>
</tr>
<tr>
<td>4</td>
<td>Flow-through cell</td>
<td>Formulations with limited solubility drugs</td>
</tr>
<tr>
<td>5</td>
<td>Paddle over disk</td>
<td>Transdermal dosage forms</td>
</tr>
<tr>
<td>6</td>
<td>Cylinder</td>
<td>Transdermal dosage forms</td>
</tr>
<tr>
<td>7</td>
<td>Reciprocating disk</td>
<td>Nondisintegrating MR dosage forms</td>
</tr>
</tbody>
</table>

Out of these apparatus 1 and 2 are the most widely used dissolution test apparatus for testing of oral solid dosage forms. The reproducible performance of these apparatus is assured by performing calibration at specified time intervals by employing a) Chemical calibration — where the dissolution of Nondisintegrating salicylic acid tablets and disintegrating prednisolone tablets is monitored and inter-unit variation is determined; or b) Physical calibration — where the apparatus parameters like vibration, eccentricity, alignment, centering and wobble of the shaft, temperature control, agitation rate are checked.

On the other hand, selection of certain dissolution test parameters (media, volume and composition, temperature and agitation) which will assure the complete release of the drug and discriminate various batches of formulations with respect to rate of release, is very important for the quality control of IR formulations. The common requirements for such tests suggested in various pharmacopoeias include:

- Sink conditions (highest dose should achieve approximately 30% of solubility concentration to assure that dissolution is not significantly influenced by solubility characteristics)
• Media. Aqueous (pH range 1.2 to 8) (to correspond with the physiologic pH in different parts of GI tract)
• Agitation intensity. Slow (50 to 100 rpm, apparatus 1 or 2) (to ensure sufficient discriminatory power to the dissolution test)
• Volume  500 to 1000 ml (in some cases sufficient enough to ensure 'sink condition')
• Temp. 37 ± 0.5°C (corresponding to normal body temperature)

However, such test conditions may not always provide the information on the biopharmaceutical characteristics i.e. performance of the formulation under physiological conditions after administration.

Similarly for quality control of modified release formulations of different drugs (with different solubility behaviours), various test media are preferred by official guidelines/pharmacopoeias. These are given below (Table 1.2) :

**Table 1.2**

<table>
<thead>
<tr>
<th>Test parameter</th>
<th>Description</th>
<th>Pharmacopoeia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of medium</td>
<td>500 – 1000 ml</td>
<td>FIP, Ph. Eur.</td>
</tr>
<tr>
<td>pH</td>
<td>pH 1 – 6.8, water with justification</td>
<td>FIP, FDA Ph. Eur</td>
</tr>
<tr>
<td></td>
<td>pH 1 – 7.6, water with justification</td>
<td>Ph. Eur</td>
</tr>
<tr>
<td>Additives</td>
<td>Enzymes, surfactants, salts</td>
<td>FIP FDA Ph. Eur</td>
</tr>
<tr>
<td></td>
<td>1% SLS possible</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low concentrations of surfactant</td>
<td></td>
</tr>
<tr>
<td>Deaeration</td>
<td>Product-by-product validation</td>
<td>FIP Ph. Eur</td>
</tr>
<tr>
<td></td>
<td>Mandatory for flow through apparatus</td>
<td></td>
</tr>
</tbody>
</table>

**Dissolution test specifications:**

Once the dissolution apparatus and test conditions are selected, one has to set the criteria of drug release, dissolution test specifications, from a particular formulation under test, as the quality parameters. Based on the number of specifications, the dissolution test can be referred as a) single-point dissolution test (one dissolution specification) or b) multi-point dissolution test (more than one dissolution specifications).

**Single-point dissolution testing**

In case of single-point dissolution testing amount of drug released at a particular time point is determined and specifications for that time (e.g. NLT 85% in 15 min) decides the acceptable or unacceptable release behaviour of the formulation.

Such a test is frequently recommended QC test for IR oral solid dosage forms. However, it is applicable only for QC test of a highly soluble and rapidly dissolving drug products
and can be considered adequate for biowaiver for rapidly dissolving products containing highly soluble and highly permeable drugs

Multi-point dissolution test\textsuperscript{16} - Characterisation of complete dissolution profiles -
In case of multi-point dissolution studies, release of a drug is monitored as a function of time i.e. amount of drug released at more than one time points is monitored. Multi-point dissolution study is also termed as dissolution profile study. The specifications for release at different time points characterise the release behaviour of the formulation throughout the course of dissolution testing. Such tests and their specifications are applicable in following cases -

- For establishing quality of MR products
- To define in vitro dissolution specifications for generic IR or MR products
- To waive bioequivalence requirements for lower strengths of an IR dosage form
- To establish IR products sameness after certain changes in components and composition (except for drugs with high solubility and high permeability), in batch size and in manufacturing equipment (level 2 post-approval changes of SUPAC IR\textsuperscript{17})
- To establish MR product sameness after level 3 post-approval changes of SUPAC MR\textsuperscript{18}
- To establish product sameness after level 3 post-approval changes of both IR and MR products
- In developing point-to-point correlations or predicting the entire in vivo plasma profile based on dissolution data, regardless the type of the product

Further, it has been recommended that there should be at least three specifications to characterize the drug release from a MR formulation as follows\textsuperscript{15} -

\begin{tabular}{|l|l|}
\hline
\textbf{Time and % drug release} & \textbf{Rationale} \\
\hline
1-2 h; 20-30\% drug release & to provide assurance against premature drug release/dose dumping \\
\hline
Around 50\% & to define the dissolution pattern/behaviour \\
\hline
Last sampling point, > 80\% & to ensure almost quantitative release (< 80\% release, justification) \\
\hline
\end{tabular}

Dissolution of sparingly water-soluble or water-insoluble drugs from their formulations:
For many poorly soluble drugs the rate of dissolution becomes a limiting factor for their absorption from drug formulation. Therefore, development of a meaningful dissolution test for drug products with limited water solubility has become a challenge. In case of
these drugs, both the physicochemical properties of the drug and the in vivo physiology are important for absorption after oral administration. For in vitro dissolution test it is essential to achieve adequate release of the drug from a formulation. With this aim several approaches have been adopted for dissolution testing of sparingly water-soluble or water-insoluble drugs.

Many researchers have suggested use of surfactants e.g. cationic (cetyltrimethylammonium bromide), anionic (sodium lauryl sulphate) or non-ionic (polysorbate/Tween), with the recommendation of using the lowest amount of surfactant required to solubilize the drug substance from the dosage form in order to achieve greater than 85% dissolution in a reasonable time interval (i.e. 120 min or less)\(^9\). A detailed report on application of various types of surfactants (natural like bile salts and synthetic like SLS, sodium oleate) in studying the in vitro dissolution profiles of different water-insoluble drugs (griseofulvin, carbamazepine, clifibrate, medroxyprogesterone acetate and cortisone acetate) has been published. The report recommends the use of surfactant is preferable over organic solvents for studying the aqueous dissolution of water-insoluble drug products\(^20\). Several reports have appeared in the literature indicating successful utilization of surfactants in dissolution media for dissolution testing of water-insoluble or sparingly water-soluble drugs e.g. carbamazepine\(^21\); Danazol\(^22\); piroxicam\(^23\); prazosin.HCl, hydrochlorothiazide, quinestrol and spironolactone\(^24\). The role of bile salts as wetting agents for water-insoluble drugs and application of the bile salts in studying in vitro dissolution of such drugs, have been studied\(^25\)\textendash\(^28\). On the other hand, some studies have reported successful use of biorelevant dissolution tests to achieve correlation between in vitro dissolution and in vivo bioavailability of some poorly water-soluble drugs like atovaquone, troglitazone, sanfetrinem cilextil and GV150013X\(^29\) and albendazole, danazol, atovaquone, troglitazone\(^30\).

**In vitro-in vivo correlation (IVIVC) for oral drug formulations: An overview**

**What is in vitro-in vivo correlation (IVIVC)?**

It is a predictive mathematical model describing the relationship between an in vitro property of a dosage form, usually the rate or extent of drug dissolution or release, and a relevant in vivo response e.g. plasma drug concentration or amount of drug absorbed\(^10\). To obtain an IVIVC, at least three batches of the same drug should be available which differ in their in vivo as well as in vitro performance. In case of difference in in vivo performance of these batches, in vitro test conditions can be suitably modified in order to correspond with the in vivo data of the batches. On the other hand, if the in vitro behaviour is different, it may be possible to modify test conditions to achieve similar
dissolution profiles of the batches showing similar in vivo behaviour, to establish an in vitro-in vivo correlation\textsuperscript{10}.

**Current Status of IVIVC:**

The performance of ER/SR/MR formulations as observed in in vitro test does not necessarily mean that those formulations will behave similarly in vivo\textsuperscript{31}. For example, three different ER diclofenac sodium tablet formulations showed identical in vitro dissolution profiles when tested in simulated intestinal fluid (without enzymes), but they exhibited different plasma drug levels when tested in humans\textsuperscript{32}. In vitro studies of ER solid dosage form prepared from cholesterol for delivering a model antigen indicated that about 20\% of the antigen was released within 8 h with a further release up to 15 days\textsuperscript{33}. But the same formulation could release about 60\% of the antigen in 2 days when tested in mice\textsuperscript{34}. Tandt et al\textsuperscript{35}, have demonstrated that the dissolution test conditions for indomethacin ER formulations (USP Apparatus 1, pH 6.2) failed to discriminate a commercial product and a test product. The in vitro dissolution profiles were found to be similar but the test formulation showed longer lag time, lower C\textsubscript{max} and delayed T\textsubscript{max} indicating lesser absorption than the commercial indomethacin ER product. In contrast, some reports also indicate good bioavailability of the formulations having poor in vitro dissolution profiles. Al-Angary et al\textsuperscript{36}, have reported that two commercial brands of theophylline ER formulations with significantly different dissolution profiles in simulated gastric fluid (1 h) followed by simulated intestinal fluid (11 h) (both without enzymes) were bioequivalent when tested in humans. Attempts have also been made to check predictability of disintegration time and dissolution parameters for bioavailability of some conventional tablets of naproxen. Razdan and Nagaraja have reported that these in vitro parameters failed to give true indication of bioavailability, when correlated with in vivo parameters like AUC and C\textsubscript{max}\textsuperscript{37}.

A partial list of the drugs and their formulations which have been studied for IVIVC is given in Table 1.3.
Table 1.3
List of drugs and their formulations studied for in vitro-in vivo correlation

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage form</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen (Paracetamol)</td>
<td>Tablets, Multiple unit capsules</td>
<td>38-40</td>
</tr>
<tr>
<td>Aminorex</td>
<td>Sustained release</td>
<td>41</td>
</tr>
<tr>
<td>Aminosalicylic acid</td>
<td>Tablets</td>
<td>42</td>
</tr>
<tr>
<td>Aspirin</td>
<td>Tablets, Coated tablets, capsules, timed release tablets</td>
<td>43-45</td>
</tr>
<tr>
<td>Bromocryptine</td>
<td>Modified release capsules</td>
<td>46</td>
</tr>
<tr>
<td>Cephalaxin</td>
<td>SR Tablets</td>
<td>47</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>Tablets</td>
<td>48</td>
</tr>
<tr>
<td>Chlorothiazide</td>
<td>Tablets</td>
<td>49</td>
</tr>
<tr>
<td>Chlorpheniramine maleate</td>
<td>CR formulations</td>
<td>50</td>
</tr>
<tr>
<td>Cinoxacin</td>
<td>Capsules</td>
<td>51</td>
</tr>
<tr>
<td>Deramciclane</td>
<td>Film coated tablets</td>
<td>52</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>CR matrix tablets</td>
<td>53</td>
</tr>
<tr>
<td>Dicumarol</td>
<td>Tablets</td>
<td>54</td>
</tr>
<tr>
<td>Digoxin</td>
<td>Tablets</td>
<td>55-57</td>
</tr>
<tr>
<td>Diltiazem HCl</td>
<td>SR formulations</td>
<td>58</td>
</tr>
<tr>
<td>Doxantrazole</td>
<td>Tablet, Suspension</td>
<td>59</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>Capsules</td>
<td>60</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>IR tablets</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>IR tablets (with metformin)</td>
<td>62</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>Tablets</td>
<td>63</td>
</tr>
<tr>
<td>Hydrochlorothiazide</td>
<td>Tablets</td>
<td>64</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>Modified release capsules</td>
<td>65</td>
</tr>
<tr>
<td>Iloprost</td>
<td>SR capsules</td>
<td>66</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>Microcapsules</td>
<td>67</td>
</tr>
<tr>
<td>Levosimendan</td>
<td>MR capsules</td>
<td>68</td>
</tr>
<tr>
<td>Lithium carbonate</td>
<td>Tablets, Capsules</td>
<td>69</td>
</tr>
<tr>
<td>Metformin</td>
<td>MR capsules, CR tablets</td>
<td>70,71</td>
</tr>
<tr>
<td>Methaqualone</td>
<td>Tablets, Solid dosage forms</td>
<td>72,73</td>
</tr>
<tr>
<td>Methenamine</td>
<td>Tablets</td>
<td>49</td>
</tr>
<tr>
<td>Metoprolol tartarate</td>
<td>ER tablets</td>
<td>74</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>Tablets, Capsule, Solid dispersions</td>
<td>49,75</td>
</tr>
<tr>
<td>Phenyltoin</td>
<td>Tablets</td>
<td>76</td>
</tr>
<tr>
<td>Phenyltoin sodium</td>
<td>Capsules</td>
<td>77</td>
</tr>
<tr>
<td>Prednisone</td>
<td>Tablets</td>
<td>78,79</td>
</tr>
<tr>
<td>Primidone</td>
<td>IR tablets</td>
<td>80</td>
</tr>
<tr>
<td>Propranolol, HCl</td>
<td>IR and ER formulations</td>
<td>81</td>
</tr>
<tr>
<td>Pseudoephedrine sulphate</td>
<td>SR Tablets</td>
<td>82</td>
</tr>
<tr>
<td>Pseudoephedrine HCl</td>
<td>CR Tablets</td>
<td>83</td>
</tr>
<tr>
<td>Remoxipride</td>
<td>SR coated spheres</td>
<td>84</td>
</tr>
<tr>
<td>Salbutamol sulphate</td>
<td>Immediate release, Osmotic pump</td>
<td>85</td>
</tr>
<tr>
<td>Spirolactone</td>
<td>Tablets</td>
<td>86</td>
</tr>
<tr>
<td>Sulfamethazine</td>
<td>Tablets</td>
<td>87</td>
</tr>
<tr>
<td>Sulfamethoxazole/Trimethoprin</td>
<td>Tablets</td>
<td>88</td>
</tr>
<tr>
<td>Sulfisoxazole</td>
<td>Tablets</td>
<td>76</td>
</tr>
<tr>
<td>TA-5707F</td>
<td>CR tablets</td>
<td>89</td>
</tr>
<tr>
<td>Theophylline</td>
<td>SR tablets, ER tablets</td>
<td>90,91</td>
</tr>
<tr>
<td></td>
<td>SR formulations, oral formulations</td>
<td>92, 93</td>
</tr>
<tr>
<td></td>
<td>Diffusion controlled DDS</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>Microspheres</td>
<td>95</td>
</tr>
<tr>
<td>Triple Sulfa</td>
<td>Tablet, Suspension</td>
<td>96</td>
</tr>
<tr>
<td>Zidovudine</td>
<td>Microspheres</td>
<td>97</td>
</tr>
</tbody>
</table>
Till 1987 there were no consistently meaningful IVIVCs established for the ER dosage forms\textsuperscript{10,98}. In 1988, a USP Pharmacopeial Forum stimuli article classified the IVIVCs into Levels A, B and C, based on their order of usefulness and the method used to correlate the data, which have been adopted by USP XXIII\textsuperscript{10}. A workshop on in vitro-in vivo testing and correlation for oral controlled/modified release dosage forms (1990), Washington DC, came up with a concept that development of an IVIVC should be on product by product basis\textsuperscript{99}. Various procedures for development, evaluation and application of an IVIVC were described and a concept of validation of dissolution specifications by bioequivalence study involving two batches of the product with dissolution profiles at the upper and lower dissolution specifications was suggested\textsuperscript{99}. Levels of correlation are described in detail in USP XXIII, NF XVIII 1995 and also the methods for the establishment of the dissolution specifications.

In 1993, during a USP/AAPS/FDA - sponsored workshop on scale-up of oral extended release dosage forms it was identified that the objectives of an IVIVC are to utilize dissolution as a surrogate for bioequivalence testing and to help establish dissolution specifications\textsuperscript{100}.

Thus, there is an increasing confidence in IVIVC for estimating the in vivo bioavailability characteristics of an ER drug product. But, development of an IVIVC with high predictability and identification of specific applications for such correlations is not still well defined.

A survey carried out by US FDA Centre for Drug Evaluation and Research, indicates increase in number of times the IVIVCs were developed for the new drug applications (NDA) submission from 9 IVIVCs in 60 submissions (1982-1992) to 9 IVIVCs in 12 submissions (Oct 1994 - Oct. 1995)\textsuperscript{10}.

The Schedule Y of the Indian Drugs and Cosmetics Act, 1940 and Drugs and Cosmetics Rule, 1945, describes "the Indian Regulatory requirement and guidelines on clinical trials for import and manufacture of New Drug"\textsuperscript{101}. It includes bioavailability studies and dissolution studies on oral dosage forms under the category of special studies. These are required to be submitted on the formulations manufactured in the country. Although the manufacturer has to submit the performance equivalence reports, separately for in vivo performance and for in vitro performance, it is not mandatory to establish correlation between the data generated by the in vivo testing and the in vitro testing of the formulations\textsuperscript{101}. On the other hand, establishment of IVIVC and submitting it along with the bioavailability and dissolution study data may help for the waiver in case of post manufacturing changes as per the US FDA guidance\textsuperscript{10}. 
Biopharmaceutics Classification System: Classification of drugs based on their biopharmaceutical properties

It has been established that the bioavailability – rate and extent of absorption - of a drug after oral administration depends on two fundamental factors – 1) drug dissolution and 2) permeability of the drug through GIT. Based on this concept, Amidon, Shah and co-workers, have proposed a biopharmaceutics drug classification system which provides an indication or likelihood of correlation of in vitro drug product dissolution and in vivo bioavailability\textsuperscript{102} (IVIVC). These biopharmaceutic drug classes and the likelihood to obtain IVIVC for immediate release products are summarized in Table 1.4

<table>
<thead>
<tr>
<th>Class</th>
<th>Solubility</th>
<th>Permeability</th>
<th>IVIVC expectations</th>
<th>Examples of drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>High</td>
<td>High</td>
<td>IVIVC- if dissolution rate is slower than gastric emptying rate, otherwise limited or no correlation</td>
<td>Verapamil, Propranolol, Metoprolol</td>
</tr>
<tr>
<td>II</td>
<td>Low</td>
<td>High</td>
<td>IVIVC- expected if in vitro dissolution rate is similar to in vivo dissolution rate, unless dose is very high</td>
<td>Ketoprofen, Naproxen, Carbamazepine</td>
</tr>
<tr>
<td>III</td>
<td>High</td>
<td>Low</td>
<td>Absorption (permeability) is rate determining and limited or no correlation with dissolution rate</td>
<td>Ranitidine, Cimetidine, Atenolol</td>
</tr>
<tr>
<td>IV</td>
<td>Low</td>
<td>Low</td>
<td>Limited or no IVIVC expected</td>
<td>Furosemide, Hydrochlorothiazide</td>
</tr>
</tbody>
</table>

Note: Here a limited correlation means that the dissolution rate while not rate controlling may be similar to the absorption rate and the extent of correlation will depend on the relative rates.

It can serve as basis for setting in vitro dissolution specifications and prediction of likelihood to achieve successful IVIVC

Further, Sievert and Siewert proposed likelihood of obtaining IVIVC for MR formulations of various drugs belonging to class I to class IV of BCS\textsuperscript{15} (Table 1.5)
Table 1.5
Likelihood of IVIVC for MR formulations:

<table>
<thead>
<tr>
<th>BCS Class</th>
<th>Solubility</th>
<th>Permeability</th>
<th>IVIVC expectations</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>High</td>
<td>High</td>
<td>IVIVC expected because dissolution rate is slower than gastric emptying rate</td>
</tr>
<tr>
<td>II</td>
<td>Low</td>
<td>High</td>
<td>IVIVC expected for similar in vitro and in vivo dissolution rates</td>
</tr>
<tr>
<td>III</td>
<td>High</td>
<td>Low</td>
<td>Absorption (permeability) is rate determining, therefore, limited or no correlation with dissolution rate</td>
</tr>
<tr>
<td>IV</td>
<td>Low</td>
<td>Low</td>
<td>Limited or no IVIVC expected</td>
</tr>
</tbody>
</table>

Recently based on the biopharmaceutical characteristics of the drug (BCS class) US FDA issued a guidance on waiver of in vivo bioavailability and bioequivalence studies for immediate release dosage forms containing certain active moieties/active ingredients based on Biopharmaceutics Classification System\textsuperscript{103}. This guidance proposes that the waiver for bioavailability and bioequivalence studies can be granted for the immediate release formulations containing BCS class I drugs (high solubility and high permeability drugs) presented in a fast dissolving form. It defines the terms high solubility, high permeability and fast dissolving IR formulations as follows –

**Highly soluble drug:** A drug substance is considered highly soluble when the highest dose strength is soluble in 250 ml or less of water over the pH range of 1 to 7.4.

**Highly permeable drug:** A drug substance is considered highly permeable when the extent of absorption in humans is determined to be >90% of an administered dose based on mass balance determination, or in comparison to an intravenous reference dose in the absence of evidence suggesting instability in the gastrointestinal tract. Other methods, like intestinal permeability studies, can also be used to determine the permeability of the drugs.

**Rapidly dissolving IR formulation:** An IR drug product is considered to be rapidly dissolving when NLT 85% of the label amount of the drug substance dissolves in 30 min using USP apparatus 1 (basket) at 100 rpm (or apparatus 2, paddle, at 50 rpm) in a volume of 900 ml, or less, in each of the following media – 1) acidic media, such as 0.1 N HCl or SGF USP without enzymes; 2) 1 pH 4.5 buffer and 3) a pH 6.8 buffer or SIF USP without enzymes.

The greatest potential benefits of BCS waivers are seen in case of – a) drugs where human exposure is highly undesirable, b) long half-life drugs with high inter-subject variability, c) difficulty in d'recruiting subject population, d) difficulty in bioanalysis and e) endogenous compounds\textsuperscript{104}. Further, based on the BCS classification a simple approach for developing in vitro dissolution test has been proposed by Dressman\textsuperscript{3} which involves the following steps –
1. Classify the drug substance according to BCS
2. Choose an appropriate medium –
   Class I and III – use most simple but reliable medium possible, addition of surfactant is unnecessary (e.g. SGF, SIF without enzymes)
   Class II and IV – biorelevant dissolution media (details are discussed in later section) warranted
   For IVIVC – SGF (without enzyme) plus surfactant, FaSSIF (fasted state), Ensure, Milk, FeSSIF (fed state)
   For QC – SGF/SIF without enzymes plus surfactant
3. Choose an appropriate volume – in general, volumes for the fasted state will be lower than volumes for fed state
4. Choose an appropriate test duration and sampling times –
   Class I and III – short test (up to 30 min) with single-point sampling to verify that appropriate amount has dissolved
   Class II and IV – test duration depends on the region of gut permeable to the drug and whether the drug is to be administered in a fasted or fed state; multiple sampling required to define the dissolution profile
5. Apparatus: USP apparatus 2 (paddle) for IR products unless there is a strong reason for another type of dissolution tester
6. Agitation (rpm) • 50 or 75 rpm
   (in case of coning, 100 rpm or use of peak vessel; in case of capsules, prevent floating with a sinker)

Inspite of its tremendous potential to address biopharmaceutical issues of several drugs BCS has following limitations -
   a) It is based on the conservative approach that drug absorption occurs preferentially in the upper part of the GIT (for permeability and solubility evaluations)\textsuperscript{105}.  
   b) Its applicability to the conception of in vitro methods is limited because dissolution remains unaffected as far as in vitro dissolution experiments are performed under 'sink' conditions (complete release of the drug from an individual dosage form results in a concentration not exceeding 30% of its maximum solubility)\textsuperscript{106}.  
   c) It assesses solubility and permeability and not dissolution and bioavailability directly, leading to difficulty in interpretation in terms of kinetic parameters\textsuperscript{106}.  
   d) It can not be applied to drugs undergoing presystemic metabolism, degradation in GIT, etc.  
   e) It can not be applied to racemic drugs showing enantiomer-specific pharmacokinetics.
Types of IVIVC:

Broadly the in vitro-in vivo correlations are classified as -

1. Pharmacological correlations – based on clinical observations
2. Semi-quantitative correlations – based on blood levels/urinary excretion data
3. Quantitative correlations – based on absorption kinetics

Levels of correlation -

US FDA guidance to industries on extended release oral dosage formulations gives good account of different levels of correlation\(^\text{10}\) -

**Level A** correlation is the highest category of correlation. It is a point-to-point relationship between the in vitro data and the in vivo data. The data treatment involves Wagner-Nelson method or Loo-Reigleman method or deconvolution followed by comparison of fraction of the drug absorbed and the fraction of drug dissolved in vitro to obtain a linear correlation. The dissolution conditions in such a case can serve as surrogate for in vivo performance of the formulation and no additional human studies are needed to justify change in manufacturing site, raw material supplier or minor formulation changes. It can act as a meaningful quality control procedure predictive of the in vivo performance of the formulation.

In case of **Level B** correlation, mean in vitro dissolution time (MDT\(_{\text{in vitro}}\)) is compared with mean in vivo residence time (MRT) or mean in vivo dissolution time (MDT\(_{\text{in vivo}}\)). Thus, although all the data obtained from in vitro and in vivo studies is utilized for establishing correlation, there is no point-to-point relationship. The data treatment involves Statistical moments analysis. This type of correlation does not uniquely reflect actual in vivo behaviour of the formulation, because a number of different in vivo profiles will produce similar MRT values. This has a very limited use in formulation development.

**Level C** correlation involves a single point relationship between dissolution test data and bioavailability of the drug (e.g. \(t_{50\%}\) in vitro or \% drug dissolved in 4 h and AUC/C\(_{\text{max}}\)/t\(_{\text{max}}\)). It does not utilize all the data and hence, cannot reflect the complete plasma concentration-time curve. Thus, it can only serve as guide in formulation development or as a production quality control procedure.

**Multiple level C** correlation relates more than one pharmacokinetic parameters of interest to the amount of drug dissolved at several time points of the dissolution profile. Indirectly, if a Multiple level C correlation exists there is a possibility of Level A correlation. Therefore, in such cases Level A correlation is to be sought.
Approaches to seek correlation between in vitro dissolution data and in vivo performance of the formulation:

Various approaches have been adopted to establish the in vitro-in vivo correlation. The use of various mathematical\textsuperscript{107-110}, statistical models\textsuperscript{38,110-113}, optimization techniques\textsuperscript{65} as well as computer softwares\textsuperscript{109,111,114-118} has been reported. Different methods for evaluating and correlating in vitro dissolution parameters with some in vivo parameters have been published. Drewe and Guitard\textsuperscript{46} have applied various methods to establish in vitro-in vivo correlation for different bromocryptine MR capsules, as follows:

a. Comparison of cumulative absorption profile and cumulative in vitro dissolution profile.

b. Correlation of corresponding times to dissolve and respectively, absorb the same fraction of the dose (for approximately 80-100 % of the dose).

c. Correlation between first order dissolution rates and respective bioavailability data by plotting in vitro dissolution rate constants vs relative AUC (good linear correlation, $r = 0.994$).

d. Correlation of cumulative percent dissolved vs (time)$^2$.

e. Correlation between in vitro mean dissolution time and in vivo mean dissolution time (weak linear correlation, $r = 0.91$).

Rekhi and Jambhekar\textsuperscript{81} calculated fraction of drug absorbed at a given time for propranolol hydrochloride extended release bead products and correlated it with fraction of drug released in vitro and the corresponding times.

Liu, et al\textsuperscript{119} have proposed a method for analysis of the IVIVC of ER formulations. This method utilizes incremental values of dissolved or absorbed fractions of the drug, instead of the cumulative fractions released or absorbed, to construct a $\chi^2$ for demonstrating the in vitro-in vivo similarity of an ER product. These $\chi^2$'s enable comparison of different in vitro dissolution profiles of a product to come to an appropriate dissolution profile representing the in vivo release pattern of the product.

USP XXIII\textsuperscript{12} gives different levels of correlation (level A, B and C\textsuperscript{99}) which utilize various mathematical and statistical techniques for establishment of in vitro-in vivo correlation such as convolution and deconvolution method, statistical moment analysis and single point comparison method. US FDA guidance to industries on extended release oral solid dosage forms\textsuperscript{10} indicates multiple level C correlation along with the correlation levels described in USP XXIII.

The most commonly used methods include comparison of fraction absorbed in vivo and fraction released in vitro at given times (Wagner-Nelson method/deconvolution method)\textsuperscript{38,83,84,120} and statistical moment analysis\textsuperscript{89}. 
Statistical moment approach (mean time parameters) can facilitate correlation between in vitro dissolution parameter and bioavailability parameter and in turn, help to predict bioavailability of the formulation by monitoring its dissolution profile\textsuperscript{121,122}.

Brazzell and Kaplan\textsuperscript{121} pointed out that accuracy of the results and meaningful conclusions using statistical moment analysis are dependent on the design of the study from which the data is generated. Thus, expansion of sampling schedule to longer times can help to minimize the impact of error in the determination of elimination rate constant inherent due to biological and analytical variability. Finally the optimum information, in terms of estimates of MATs and MDTs, can be generated using frequent sampling during absorption phase and adequate sampling during the terminal elimination phase to minimize impact of extrapolation error on the estimate of elimination rate constant.

Block and Banakar\textsuperscript{122} have demonstrated the utility and inherent simplicity of this model independent approach to IVIVC by transforming the data possessing poor correlation between in vitro and in vivo parameters. They have applied Mean Time concept based on statistical moments for IVIVC to the in vitro and in vivo data generated by McNamara, et al., for six furosemide tablet formulations using both plasma concentration-time data and urinary excretion data. There was satisfactory improvement in the correlation between MRT\textsubscript{in vitro} and MRT\textsubscript{in vivo} after transformation of the data using statistical moments with correlation coefficient 0.99 and 0.98 at $p<0.05$ when urinary excretion data and plasma concentration data was used, respectively.

Further, a concept of continuous IVIVC (CIVIVC) has been suggested by Brockmeier\textsuperscript{105} to quantify the degree to which in vitro dissolution results reflect the in vivo dissolution process by using in vitro and in vivo data of a single formulations (as opposed to at least 3 for Level B/C correlations).

**Determination of correlation and related calculations:**
In vitro dissolution data can be utilized for prediction of in vivo performance of the dosage form if there exists a meaningful method for transformation of data. In vitro data cannot be compared directly with in vivo data since measurement of in vivo release/absorption is not straightforward. Even use of classical single point pharmacokinetic parameters such as $C_{\text{max}}$ and $T_{\text{max}}$ to assess bioavailability/bioequivalence of ER dosage forms is controversial\textsuperscript{123}. Various mathematical models and equations have been described in literature for conversion of directly measurable pharmacokinetic data to release/absorption characteristic of drug from the dosage form for comparison with in vitro data, e.g., Wagner-Nelson model\textsuperscript{124}, Moment analysis\textsuperscript{122}, Deconvolution\textsuperscript{120,125,126}. Thus, using a meaningful method for transformation of data, one can correlate in vitro dissolution data with in vivo
bioavailability data. Further, once the correlation between in vitro dissolution and in vivo performance (IVIVC) of a formulation is established, dissolution data can be utilized for the prediction of in vivo performance of the dosage form.

The data obtained from the in vivo studies (plasma concentration vs time data) is used to calculate the amount of drug absorbed (cumulative relative fraction absorbed) into systemic circulation using either Wagner-Nelson method \(^{83,102,110}\) (which considers body as a single compartment) or mathematical deconvolution method \(^{10,122,124}\) (which needs plasma concentration - time data for a fast releasing formulation e.g. intravenous (i.v.)/fast releasing oral formulation like solution or suspension or tablet for comparison). These methods utilize all the in vivo and in vitro data available which is essential for development of Level A correlation. For Level B correlation, some mean parameters like MRT or MDT\(_{\text{in vito}}\) and MDT\(_{\text{in vivo}}\) are compared \(^{1,123}\). Although all the in vivo and in vitro data is being used in this kind of correlation, it can not be a point to point correlation. And in case of level C correlation, particular in vivo parameter \((C_{\text{max}}/T_{\text{max}}/\text{AUC}/T_{1/2})\) for formulations with different in vitro dissolution behaviour are correlated with specific in vitro dissolution parameter \((T_{90}/T_{50}/\text{per cent dissolved in 45 min})\) and equation for the correlation is established \(^{1,10,127}\).

Makoid, Banakar and Dufoure have reviewed several approaches for modelling of dissolution profiles (in vitro as well as in vivo) of controlled release systems \(^{128}\). As the success of any drug delivery system is governed by the drug absorption performance which is a function of the drug release, assuming relatively fast absorption following drug dissolution, it is important to select proper model for assessing the dissolution behaviour and hence the in vivo performance of that particular formulation. It has been pointed out that although several model equations might be useful in the evaluation of the dissolution as well as absorption profiles, the least complicated model which reflects the data to the level necessary for predictions should be selected \(^{128}\).

Cumulative relative fraction absorbed (CRFA) can be calculated by using the Wagner-Nelson method from the following equation and correlated to the cumulative fraction dissolved in vitro \(^{10,83,124}\)

$$\text{CRFA} = \frac{(C_t + K_{el} \cdot AUC_{0-t}) / (K_{el} \cdot AUC_{0-\infty})}{...} \quad 1$$

where \(C_t\) is the plasma drug concentration at time \(t\), \(K_{el}\) is the elimination rate constant, \(AUC_{0-t}\) is the area under the curve from time zero to time \(t\) and \(AUC_{0-\infty}\) is the area under the curve from time zero to time infinity.

Convolution method \(^{10}\) predicts plasma drug concentrations using a mathematical model based on the convolution integral as given in the following equation,

$$C_t = \int_0^t C_0 (t-u) r_{abs} (u) \, du \quad \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots 2$$
where, $C_t$ is the plasma drug concentration resulting from the absorption rate constant ($r_{abs}$). $C_s$ is the concentration time course that would result from the instantaneous absorption of a unit amount of drug and can be estimated from either i.v bolus data, oral solution, suspension or rapidly releasing (in vivo) dosage forms.

Deconvolution method$^{10,125}$ estimates the time course of drug input (usually in vivo absorption or dissolution) using a mathematical model based on the same convolution integral as given in equation 2.

Mean residence time (MRT)$^{1,10,122}$ (or mean transit time) is the mean time for which the drug resides in the body and is calculated by using the equation

$$\text{MRT} = \frac{\text{AUMC}}{\text{AUC}} \quad \text{................................ 3}$$

where AUC is the area under the plasma concentration time curve and AUMC is the area under the moment curve (area under (plasma concentration x time) vs. time curve).

Mean absorption time (MAT) is the mean time required for the drug to reach the systemic circulation from the time of drug administration and is calculated as

$$\text{MAT} = \text{MRT}_{\text{oral}} - \text{MRT}_{\text{IV}} \quad \text{................................ 4}$$

Mean in vitro dissolution time ($\text{MDT}_{\text{in vitro}}$) indicates the mean time for the drug to dissolve under in vitro dissolution conditions and is determined using the following equation,

$$\text{MDT}_{\text{in vitro}} = \frac{\int_0^\infty (M_{\infty} - (t)) \, dt}{M_{\infty}} \quad \text{................................................. 5}$$

Finally, mean in vivo dissolution time reflects mean time for drug to dissolve in vivo and is calculated using the equation,

$$\text{MDT}_{\text{solid}} = \text{MRT}_{\text{solid}} - \text{MRT}_{\text{solution}} \quad \text{................................................. 6}$$

i.e. $\text{MDT}_{\text{in vivo}} = \text{MRT}_{\text{solid}} - \text{MRT}_{\text{solution}}$

**Development and evaluation of IVIVC:**

The US FDA guidance$^{10}$ to industries on extended release oral dosage formulations provides guidelines for development of an IVIVC for a product to support the application for biowaiver. In general, the in vivo and in vitro studies should be conducted under following considerations -

- **In vivo studies -** Human bioavailability studies should be carried out in a population of 6-36 subjects in a cross-over or parallel design, under fasted condition (or under fade state if drug not tolerated empty stomach).
- **In vitro dissolution studies -** For in vitro drug release test the preferred apparatus are USP apparatus 1 at 100 rpm or USP apparatus 2 at 50-75 rpm or USP apparatus 3 or USP apparatus 4, if required. The aqueous medium should comprise water or buffers of pH upto 6.8. Addition of surfactants (SLS 1%) is permitted for poorly water soluble drugs. In general, nonaqueous or hydroalcoholic media are discouraged. At
least 12 units of the drug product should be tested for in vitro dissolution and %CV for mean dissolution profiles should be <10%

The correlations are preferred in the order - Level A > Multiple level C > Level C > Level B > Rank order correlation.

**Development and evaluation of IVIVC**:

Various steps involved in the development of different levels of IVIVC (Level A and C) are given below-

1. **Development and evaluation of Level A correlation.**
   For developing a level A IVIVC one has to -
   i) Develop formulations with different release rates (slow, medium, fast; or single release rate for condition independent dissolution)
   ii) Generate in vitro dissolution profiles and in vivo plasma concentration profiles
   iii) Estimate in vivo absorption/dissolution-time course by appropriate deconvolution method (Wagner-Nelson/numerical deconvolution/Loo-Reigleman method) for each formulation and subject.

   Further, it is recommended that in vitro dissolution method should discriminate between formulations and in early stages of IVIVC development, alterations in dissolution conditions to attempt 1:1 correlation are advisable

**Evaluation** –

The IVIVC developed is evaluated to demonstrate that predictability of in vivo performance of a drug product from its in vivo characteristics is maintained over a range of dissolution release rates and manufacturing changes.

Predictive performance/predictability of the IVIVC is determined in terms of prediction error,

\[
\text{Prediction error (\%PE)} = \frac{(\text{Obs. value} - \text{Pred. value})}{\text{Obs. value}} \times 100
\]

The prediction performance has been tested in two ways -

a) Internal predictability which tells 'how well the model describes the data used to define the IVIVC' (Uses initial data used to define IVIVC); and

b) External predictability which assesses 'how well the model predicts the data when one or more additional test data sets are used that differ from those used to define IVIVC' (Uses additional data set)

2. **Development and evaluation of Level C IVIVC**:

For level C correlation ‘amount of drug dissolved at various time points is correlated with \(C_{\text{max}}, \text{AUC} \) or any other suitable in vivo parameter’.  

For Multiple level C IVIVC in vitro data for at least 3 dissolution time points (early, middle and last stage of dissolution profile) is essential, which is then compared with suitable in vivo parameters at more than one time points.

Evaluation of predictability is application specific in case of either level C or multiple level C correlations.

Comparison of dissolution profiles for obtaining biowaivers:

One of the major applications of IVIVC is to obtain the biowaivers for changes in the manufacturing of a product which involve biowaivers without an IVIVC and biowaivers with IVIVC for non-narrow therapeutic window drugs as well as for narrow therapeutic window drugs. The detailed guidelines and the criteria for obtaining such waivers on bioavailability studies are given in US FDA guidance on extended release dosage forms. One of the criteria for allowing a biowaiver to waive the bioequivalence requirements for lower strengths of a dosage forms is to prove the similarity of the dissolution profiles of the batch for which the biowaiver is sought and the batch for which valid IVIVC has been established. This is achieved by comparing the dissolution profiles of these batches. For comparing the dissolution properties of several approaches have been reported in literature.

The US FDA guidance for industry on dissolution testing of immediate release solid oral dosage forms gives account of different approaches to compare the dissolution profiles like a) model independent approach using a similarity factor, b) Model independent multivariate confidence region procedure and c) Model dependent approaches. Out of these approaches a simple model independent approach which uses a difference factor \( f_1 \) and a similarity factor \( f_2 \) is widely used to compare dissolution profiles.

The difference factor \( f_1 \) calculates % difference between two curves at each time point and measures relative error between the two dissolution curves:

\[
f_1 = \frac{\left( \frac{1}{n} \sum_{i=1}^{n} \mid R_i - T_i \mid \right)}{\frac{1}{n} \sum_{i=1}^{n} R_i} \times 100
\]

where \( n \) = number of time points, \( R_i \) = dissolution value (%) of reference batch at time ‘t’ and \( T_i \) = dissolution value (%) of test batch.

While the similarity factor \( f_2 \) is a logarithmic reciprocal square root transformation of the sum of squared error and measures similarity in the % dissolution between to curves:

\[
f_2 = 50 \times \log\left\{ \left[ 1 + \frac{1}{n} \sum_{i=1}^{n} (R_i - T_i)^2 \right]^{0.5} \right\} \times 100
\]

where \( n, R_i \) and \( T_i \) are the same as in case of \( f_1 \).

This approach has tremendous advantage over single-point comparison approaches as it covers the entire course of dissolution of the product and not the dissolution at a single time point. This approach though widely accepted and recommended in the US FDA.
guidance, is sensitive to length of sampling data points. O’hara et al have reviewed various methods used to compare the dissolution profile data. Other approaches reported in the literature for comparing the in vitro dissolution profiles include Rescigno indices, model-dependent approaches and the difference in similarity (Sd) approach.

**Prediction of food effects using dissolution studies:**

Charman et al have reviewed various physicochemical and physiological mechanisms that are involved in food-induced effects on the absorption of drug emphasising the role of lipids and pH of the gastrointestinal segments. Several attempts to model food-induced effects on drug dissolution have been described in the literature, including use of the following:

- a) Bile salt and lecithin
- b) pH changes
- c) Fat emulsions such as Intralipid and milk
- d) Well-defined nutritional drinks
- e) Enzymes
- f) Dynamic lipolytic models
- g) Presoaking in oil
- h) Viscosity enhancement
- i) Inert beads to obtain a grinding effect similar to that of solid food stuff

Khan has reviewed several approaches to simulate the in vivo environment in the in vitro dissolution. Aoki et al have modified the paddle method by inserting the polystyrene beads to simulate the GI motility and mucin plugs after administration of phenylpropylamine hydrochloride matrix tablets into fasted beagle dogs. They observed that the modified paddle method showed better IVIVC as compared to the conventional paddle method.

El-Arini et al attempted to simulate the pH of the GI tract and the food induced changes to the bioavailability of theophylline from beads either embedded in the tablet or filled in the capsule. They inserted a dialysis cell containing the dosage form in a small volume of fluid in the dissolution medium in a dissolution vessel and the physiological conditions were simulated by adjusting the fluid of dialysis cell. This has enabled the testing of ER formulations under various food-induced conditions.

Macheras et al, have used milk with various levels of fat content as the dissolution medium and demonstrated direct relationship between fat contents of milk and the dissolution data for theophylline matrix tablet or capsule formulations. They could achieve good correlation between in vivo data obtained after administration of these
formulations in humans after high fat milk and the dissolution data obtained using 7.5% fat content milk as the dissolution medium.

Maturu et al\textsuperscript{141}, have simulated the effect of high fatty food on the in vivo behaviour of theophylline matrix tablets and beads filled in capsules by treating the dosage form (or contents) in peanut oil for 2 h prior to standard dissolution testing. The dissolution data, thus obtained, showed good correlation with in vivo percent dissolved in humans after high fat breakfast.

**Biorelevant dissolution testing: Simulation of in vivo environment in the in vitro dissolution test:**

In case of oral solid dosage forms specific dissolution conditions are established after studying thoroughly the environment in the gastrointestinal tract to which the formulation is subjected and attempts are, thus, made to simulate these conditions as close as possible\textsuperscript{147}. Dressman et al have reviewed comprehensively the physiological data relevant to dissolution testing of IR products and the circumstances i.e. the dissolution test conditions, under which one can use dissolution test as a tool to predict in vivo performance of a formulation\textsuperscript{147}. Such dissolution test conditions are generally referred as 'Biorelevant dissolution conditions'. It is important to understand physiology of the gastrointestinal tract to build on a 'Biorelevant dissolution test'. Some of the characteristic features of different parts of GIT\textsuperscript{148} are given in Table 1.6.

**Table 1.6**

<table>
<thead>
<tr>
<th>Segment</th>
<th>Function</th>
<th>Size (diameter x length)</th>
<th>Surface area (m\textsuperscript{2})</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>Digestion of foods</td>
<td>15 cm x 20 cm</td>
<td>3.5</td>
<td>1-3.5</td>
</tr>
<tr>
<td>Duodenum</td>
<td>Neutralisation of acids</td>
<td>3-5 cm x 20-30 cm</td>
<td>2.0</td>
<td>4-6.5</td>
</tr>
<tr>
<td>Jejunum</td>
<td>Absorption of nutrients</td>
<td>3-5 cm x 240 cm</td>
<td>180</td>
<td>5-7</td>
</tr>
<tr>
<td>Ileum</td>
<td>Absorption of nutrients</td>
<td>3-5 cm x 360 cm</td>
<td>280</td>
<td>6-8</td>
</tr>
<tr>
<td>Colon</td>
<td>Absorption of water</td>
<td>3-9 cm x 90-125 cm</td>
<td>1.3</td>
<td>6-8</td>
</tr>
</tbody>
</table>

However, it was observed that several attempts made to simulate food-induced effects on bioavailability of drugs, cited above, have had extremely limited success in prediction. Therefore, FIP guidelines have incorporated dissolution test media that may reflect gastric conditions (fasted) and intestinal conditions (fasted/fed), reported earlier by Galia et al\textsuperscript{149}, which may give additional information of food-induced effects on absorption of drugs\textsuperscript{150}. The compositions of these media are given in the Table 1.7.
Table 1.7
Dissolution test media which may reflect gastric conditions (fasted) and intestinal conditions (fasted/fed)

<table>
<thead>
<tr>
<th>SGF (Fasted gastric conditions)</th>
<th>FaSSIF (Fasted intestinal conditions)</th>
<th>FeSSIF (Fed intestinal conditions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCl 0.01-0.05 N 2.5 g Sodium lauryl sulphate 2.0 g Sodium chloride Distilled water</td>
<td>KH₂PO₄ NaOH Na-taurocholate Lecithin KCI Distilled water 0.029 M qS pH 6.8 5 mM 1.5 mM 0.22 M qS 1000 ml</td>
<td>Acetic acid NaOH Na-taurocholate Lecithin KCI Distilled water 0.144 M qS pH 5.0 15 mM 4 mM 0.19 M qS 1000 ml</td>
</tr>
</tbody>
</table>

Recently several studies have been reported which have employed biorelevant dissolution conditions to assess the prediction of either in vivo performance of different drugs and formulations or food-induced effects on bioavailability of some drugs. Galia et al., studied in detail behaviour of different BCS class I (acetaminophen, metoprolol) and BCS class II (danazol, mefenamic acid, ketoconazole) drugs and their IR formulations using various dissolution media (water, SGF, SIFsp, FaSSIF, FeSSIF and milk) with the aim to predict the in vivo behaviour of these formulations. It has been concluded that FaSSIF and FeSSIF media could correlate better the dissolution behaviour and the bioavailability for class II drugs. Apart from this, recently several studies have been reported which employed biorelevant dissolution conditions to correlate the in vitro dissolution behaviour with in vivo performance of several formulations. These include glibenclamide formulations; troglitazone, atovaquone, sanfetrinem cilexetil, GV150013X, poorly soluble weak bases like dipyridamole, BIMT 17 BS, BIBU 104 XX; St John’s wort products. Klein et al., have recently reported simulation of passage of different mesalazine products through the GI tract by using USP apparatus 3 (reciprocating cylinder) by employing a pH gradient method based on mean physiological pH values in each segment of the GI tract.

In an interesting study, Al-Bahaisi et al have reported in vitro simulation of food effect on dissolution of deramicline film coated tablets by using artificial gastric juice (fasting state and fed state). They found a very good correlation between the in vitro dissolution data and the in vivo data in healthy volunteers.

Rekhi and Jambhekar have found that change-over dissolution medium (simulated gastric fluid for 1 h and simulated intestinal fluid for 11 h) gave better IVIVC than the data obtained using distilled water as the dissolution medium for propranolol hydrochloride ER bead products prepared using aqueous polymeric dispersions.

Tandt et al., studied dissolution of marketed theophylline ER formulations (Theodur® and Retafyllin) at different pH conditions (pH 3, 4, 5, 6, 6.8 and 7.5). They simulated the...
In vivo performance of these formulations using biorelevant technique. It was observed that simulated profiles obtained from dissolution data using pH 6.0 buffer were superimposable with the actual in vivo profiles of Theodur® but for Retafyllin, the data obtained using pH 4.0 buffer as the dissolution medium served the purpose well. The effect of addition of different solubilizers into the dissolution medium on in vitro dissolution of drug having poor aqueous solubility has been studied by Abrahamsson et al. They have demonstrated that addition of solubilizer in the dissolution medium provided the data with a good IVIVC for felodipine matrix tablet formulations but have cautioned that choice of solubilizer affects the results.

The dissolution test conditions have been modified by adding solubilizer in the dissolution medium and a stationary basket to hold the dosage form above the paddle to achieve reproducible hydrodynamic conditions for felodipine (a model drug having poor water solubility) matrix tablet formulation. This resulted into the dissolution data with a good IVIVC and this method was capable to discriminate the formulations with different in vivo performance.

In recent times, much effort has gone into establishing the IVIVCs for different types of MR formulations and utilize them for the development of optimum formulation. Various attempts are being made to establish the correlation either by developing discriminating in vitro dissolution tests, biorelevant dissolution test conditions or identifying the biorelevant dissolution conditions by systematic study of different physicochemical properties of the in vivo environment and trying to simulate those, in vitro, followed by comparison of the in vitro release profiles with the in vivo absorption profiles. Some workers have utilized well established pharmacokinetic parameters and the in vitro dissolution study data to predict the in vivo behaviour of the formulation. Simulation of GI tract conditions may help to understand the fate of the drug administered in a particular dosage form by oral route beforehand and thus, help to develop the optimum formulation. In this regard Abuzarur-Aloul et al. have thoroughly studied various in vitro dissolution test parameters like agitation, temperature, osmolality and polarity of the dissolution medium and their effect on the dissolution of remoxipride from ER-coated spheres to arrive at critical dissolution test conditions which reflect the in vivo behaviour of the formulations in terms of a level A correlation. They have also investigated the effect of different in vitro variables like agitation, pH, osmolality, viscosity and the presence of the bile salts on the dissolution rate of paracetamol from the formulation and established an IVIVC for multiple-unit capsules of paracetamol.

Thus, biorelevant dissolution tests, inspite of the complexity involved and several issues which need further refinement, offer a very promising in vitro tool for predicting the in vivo performance of drugs with dissolution limited absorption.
Applications of IVIVC:
In vitro – in vivo correlation is used -
- As a developmental tool for series of dosage forms to achieve desired physiological performance (Safety and efficacy)
- For evaluation of different lots of formulations as a ‘Quality control check’ to ensure desired physiological performance over desired period
- To establish the dissolution test as a surrogate for human BE studies - to reduce number of BE studies during pre-approval process and with certain scale-up and post-approval changes
- To obtain biowaivers for changes in the manufacturing
- Setting dissolution specifications
- To add in vivo relevance to in vitro dissolution specifications beyond batch-to-batch QC and thus, to make in vitro dissolution test a meaningful predictor of in vivo performance of the formulation

Limitations of IVIVC: Inspite of its wide applicability IVIVC has some limitations, as follows -
- It is difficult to achieve IVIVC in case of drugs undergoing extensive metabolism, presystemic metabolism, prodrugs, and drugs with gastric emptying dependent BA
- Considerable inter- and intra-subject variability in the pharmacokinetic behaviour of a drug limits the success of meaningful IVIVC.
- Selection of inadequate in vitro dissolution conditions without considering physicochemical properties of the drug lead to failure of the IVIVC
- It becomes more complicated if rapid dissolution is not expected due to possible toxicity or undesirable symptoms.
- It is considered to be specific for a particular formulation or product – product-specific IVIVC.

Unexplored areas:
To date lot of work has been done to utilize IVIVC to provide robust in vitro models with physiological meaning. However, some of the areas of its application need to be explored or need further research. They are -
- Applicability of different types of correlations on case-by-case basis
- Development of IVIVC in case of chiral drugs, drugs which are unstable in GI environment or which exhibit pH-dependent dissolution behaviour
- Development of IVIVC in terms of robust modelling and statistical methods
Cases where IVIVC fails to predict in vivo performance (False positive and false negative - dissolution tests)

Validation of IVIVC to avoid failure in prediction

IVIVC capable of setting dissolution specifications consistent with bioequivalence and to changes observed in the stability of the product

Exploration of possibilities to generalize IVIVC to be relevant across the formulations and technologies within some combination of drug/ product category which will require establishment of larger and stronger database

Future trends in the field:

Many laboratories are engaged to find better means to predict in vivo behaviour of the drug after oral administration by using simple in vitro dissolution tests. Efforts are on to modify the dissolution specifications to surrogate the bioavailability and the in vivo testing. Several computer programmes have been developed to simulate in vivo release pattern of the dosage forms by using the data obtained from the in vitro dissolution of the formulations or to help for development of an IVIVC\textsuperscript{109,111,114-118,160,161}. Various approaches are adopted to achieve better IVIVC (linear models, nonlinear models and other statistical models)\textsuperscript{30-33,42}. Even for immediate release formulations, in new biopharmaceutical classification of the drugs based on the solubility and permeability of the drugs, the chances to achieve good IVIVCs have been studied thoroughly\textsuperscript{20}. Newer concepts, like validation of the IVIVC for its reliability to estimate the in vivo behaviour of the formulation, have emerged\textsuperscript{10,162,163}. Applicability of Artificial Neural Networks (ANNs) towards IVIVC of ER formulations has been tested\textsuperscript{110,112,164,165}. ANNs have the potential to be used as reliable predictive tool that may overcome some of the difficulties associated with classical regression methods (especially, difficulty of providing prior specification of the regression equation structure). This may demonstrate higher degree of reliability in the predicted in vivo behaviour of the formulations\textsuperscript{164}.

There is considerable interest in extending the concept of IVIVC to nonoral dosage forms and novel drug delivery systems\textsuperscript{166}.

Conclusions:

IVIVC provides a well-founded rational tool for the development of oral formulations. It is a dynamic process running through an integrated formulation development, analytical testing and in vivo characterization of the formulation. The techniques, methods and approaches involved in development and application of IVIVC need to be constantly revised or critiqued. Ideal in vitro dissolution test with a balance between its potential as a discriminatory quality control tool and as an in vitro model for predicting
biopharmaceutical behaviour of drugs (based on a meaningful IVIVC) is the need of the hour. There is a need to explore different areas to further strengthen applicability and general acceptance of IVIVC by developing robust in vitro dissolution tests to reduce the extensive, expensive and time-consuming animal studies as well as human bioavailability/bioequivalence studies.