Know your Curcumin Bioavailability better than before …!!!

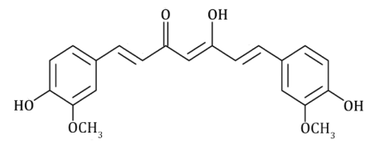
By Dr. Krishnakumar IM , Akay Flavours & Aromatics Pvt Ltd, Cochin, INDIA
Krishnakumar.IM@akay-group.com

The significance and necessity of an article on the current understanding about curcumin bioavailability issues and the methods of its determination was deeply felt during my recent interactions with various groups of people including Medical doctors, Nutritionists, Pharmacists, and Formulators. Thanks to the intense research by hundreds of universities and thousands of publications regarding the safety, mechanism of action, and health beneficial pharmacological effects of curcumin. Today, everyone is aware about the hallmarked yellow pigment in the rhizomes of the kitchen spice turmeric (*Curcuma Longa L*) as the most bioactive constituent responsible for the multitude of health beneficial effects.

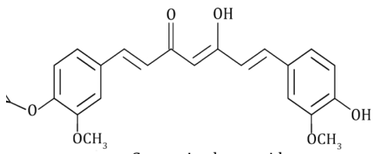
However, the seed of confusion germinates and grows fast when someone come across with the blogs and marketing claims about curcumin and its bioavailability. Some of the overenthusiastic claims as a miracle *'drug'* or *'magic bullet'* even for 'hair-growth' and 'fertility' issues invited severe criticisms. In a recent review, Nelson et al., narrated curcumin as 'a missile that continually blows up at the launch pad, and never reaches its intended target' (*Nelson et al., 2017*). But, it has been immediately defended by a group of eminent scientists through a corresponding note to the journal *"Nature"* about the fact that curcumin can modulate the oxidative stress and inflammatory pathways involved in the pathogenesis of a number of chronic diseases, if a proper dose of curcumin can be ingested regularly in a bioactive form (*Heger et al., 2017*). Here comes the significance of *"Oral Bioavailability"* of **curcuminoids [(curcumin, demethoxycurcumin (DMC) and bisdemthoxycurcumin (BDMC)].**

Free verses Conjugated curcuminoids

Absorption and distribution of bioactive forms of any substance is crucial for its functional benefits. In the case of curcumin, the *poor absorption and the rapid biotransformation to various inactive metabolites* makes it poorly bioavailable. Recent studies from USA and Japan have shown that the major metabolite of curcumin (curcumin glucuronides) possess no anti-proliferative, anti-inflammatory and antioxidant activities (*Pal et al, 2014; Shoji et al; 2014; Choudhari et al, 2015*). Curcumin glucuronides were also demonstrated to have low membrane permeability with no ability to cross the Blood-Brain-Barrier (BBB) (*Begum et al., 2008). Thus, the real challenge in the craft of bioavailable curcumin formulation turned out to be the ability to provide significant levels of free-form of curcuminoids, rather than the conjugated curcumin glucuronides (Fig. 1).*



Free Curcumin



Curcumin glucuronide
(conjugated)

Measurement of bioavailability

Liquid chromatography coupled with electrospray ionization triple quadruple tandem mass spectrometry (LC-ESI-MS/MS) has been established as the most sensitive state-of-the-art bioanalytical method for the estimation of curcumin bioavailability. Most of the current 'bioavailable' formulations have measured their bioavailability as 'free' curcumin equivalents by treating the plasma with β-glucuronidase enzyme to convert the conjugated curcumin metabolites (curcumin glucuronides and sulfates) to free curcuminoids. Since most of these formulations are not capable of providing the free-form of curcuminoids, the observed number of folds of curcumin bioavailability is the bioavailability of curcumin glucuronides only. Prof. Liu of the University of Illinois reported that *'the current methodology of bioavailability determinations is misleading and creates a lot of confusion among the users since it does not specify the detected levels of curcuminoids as whether 'free-form' or 'conjugated forms'* (Szymusiak et al., 2016).

'FCR' – a new dimension in bioavailability

The measurement of the relative percentage of *'free verses conjugated'* curcuminoids in circulation is the new dimension in curcumin bioavailability. Employing a standardized method, Kumar et al., measured the 'free' curcuminoids ratio (FCR) as a measure of the effectiveness of a formulation for the first time (*Kumar et al., 2016*). FCR represents a direct measure of the free curcuminoids in circulation verses the glucuronide conjugates. Higher the FCR, greater is the 'free' curcuminoids delivery and hence the efficacy, especially the brain health, since only free-form is BBB-permeable. Thus, checking the 'FCR' can be a "new benchmark" to distinguish a particular formulation from other formulations based on their capacity to deliver bioactive 'free-form' curcuminoids. ….*Something more than the mere number of folds of bioavailability…!*

Tissue distribution of curcuminoids

It has already been shown that the health beneficial pharmacological activities of curcumin are due to the multi-targeted mechanism of action (pleiotropic effect) to interact with genome (DNA), messengers (RNA) and proteins. Cellular uptake at safe and pharmacologically relevant concentrations has been shown to be indispensable to achieve the same. Since conjugated metabolites (glucuronides) have no permeability, it is important to have free-form of curcuminoids in plasma, especially for brain tissue distribution. So, brain pharmacokinetics curcuminoids (brain bioavailability) can be taken as a *'litmus test'* to confirm the *'true'* bioavailability of a formulation.

Fundamentals of bioavailability studies

It is interesting to note that curcumin formulations with bioavailability claims ranging from 7 to 500-folds are currently available in the global market….! Formulations with less than 50 ng/mL plasma curcuminoids are claimed to have many fold higher bioavailability than those with >300 ng/mL curcuminoids in plasma for more than 12 h.…! Awareness on the fundamentals of bioavailability, absorption and pharmacokinetics assumes significance in this context.

Area under curve (AUC) of the plasma concentration-time plot is the primary determinant of the 'number of folds'. Everyone knows that natural curcumin contains curcumin, DMC and BDMC in an approximate ratio of 77: 15: 3 (w/w). It is true that when consume unformulated standard curcumin, the relative amounts of DMC and BDMC ingested will be significantly lower than curcumin and were not even detected in plasma. But for formulations having significant absorption, they can be detected as their glucunonides and sulfates. In such cases, one can get a relatively large number of folds of bioavailability by calculating the AUC separately for curcumin, DMC and BDMC for both unformulated curcumin and for the formulation. Though this aspect is theoretically correct, many times it can lead to abnormally high values. For example, Kumar et al., has reported that their bioavailability can be 270-folds in a similar calculation, though it has only 45.5-fold enhancement in bioavailability when calculate on the basis of total plasma concentration of curcuminids (Kumar et al., 2016).

As per the definition, the drug and its formulation has to administer at equivalent amounts to compare the bioavailability. In the case of formulations with relatively low percentage of total curcuminoids, as low as 20% (w/w) or below, the definition causes some issues. For instance, in the case of a formulation with 20% total curcuminoids, the bioavailability study has to compare with 1000 mg of formulation verses 200 mg unformulated curcumin. Obviously, the maximum observed plasma curcuminoids levels (Cmax) may be as low as 0.5 to 2 ng/mL only. In such cases, even if Cmax of the formulation is only 20 to 40 ng/mL, AUC will reflect as a 'big' number, indicating a large *'number of folds'*, irrespective of the low absorption as shown by C_{max} ….! Though theoretically correct, such 'number folds' will not be the *'true'* bioavailability, rather a reflection of the low absorption in the control (placebo) group. It has to be understood that, one can find 8 to 20 ng/mL of curcumin in plasma followed by the oral administration of 500 to 1000 mg of unformulated standard curcumin as per LC-MS/MS analysis. So, always check the placebo levels (control group) in plasma and the C_{max} of the curcuminoids of the formulation.

The elimination half-life ($t_{1/2}$) is yet another pharmacokinetic parameter usually used to claim longer duration of existence of curcumin in plasma. But, *it will be useful to check the plasma curcumin levels at $t_{1/2}$ time point to better understand its significance.* Note that a typical pharmacokinetic graph should have absorption, distribution and elimination phases, unlike a graph with plasma concentrations parallel to the time axis. Yet another factors to be noted are limit of detection (**LOD**) and limit of quantification (**LOQ**) of various metabolites including tetrahydrocurcuminoids (TC). LOQ below 1 ng/mL for molecules like TC may cause *false positives* in triple quadruple mass spectrometry.

Randomized double-blinded crossover design is the golden standard for bioavailability comparison. Thomson Reuters 'impact factor' of the journal in which the study was published is another important factor for authenticity, since many paid open access journals with *'NO' impact factor* are also available today. The number of subjects in the study is also very crucial, since curcumin has been shown to have a wide variation in metabolism rate depending on the genetic makeup of an individual. While at least 25+ subjects was considered as reliable on the basis of standard deviation (statistical significance), Kumar et al., 2016 reported the largest study so far (n = 50).

Finally, it will be worthy to note the total curcuminoids content in a formulation. Formulations with 6 to 95% curcuminoids are available in the global market place today. Usually, NANO forms and liquid micelles have low percentage of curcuminoids due to the difficulty to achieve high loading levels. Though 'nano' forms are ideal for treatment (as drugs), it is always better to have particle size above 400 nm for nutraceutical and food applications owing to the regulatory hurdles and safety reasons. Above all, there is an element of *'psychological effect'* in the customer point of view, which demands moderately high levels of curcuminoids in the formulation. For instance, 500 mg dose of a formulation with 5% curcumin will have only 25 mg than with a 40% formulation, which has 200 mg curcumin…!

In sum, the recent understanding on curcumin bioavailability and efficacy demands the delivery of 'free-form' of curcuminoids at significantly higher levels in plasma and further into tissues, with systemic absorption, distribution and elimination as proven by pharmacokinetics studies employing at least 25+ subjects and publications in high impact journals. 'FCR' index can be considered as a direct measure of free-form delivery and hence a new dimension to the effectiveness of a formulation, despite the 'big' number as bioavailability enhancement fold. Finally, 100% natural, food-grade GRAS formulations without synthetic excipients in a non-NANO form is always the best choice for Nutraceuticals.

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