

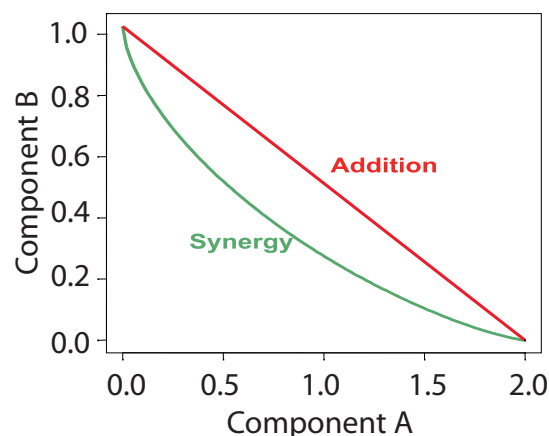
**Background:** The effects of mixtures on the chemical senses are complex and puzzling. One of these effects is called “synergy”. This term is used loosely to describe effects that cannot be explained by additivity of the components alone, and is used even more loosely to describe effects greater than “expected.” There is a lack of clarity concerning the meaning of additivity and synergy in mixture research. Can we define synergy satisfactorily and develop models to predict it? More generally, can we use data from human psychophysical studies on mixtures to draw conclusions about molecular mechanisms that occur on the tongue or at the olfactory epithelium without direct biochemical information?

**Mixtures:** Our senses are constantly exposed to chemical mixtures. Even when single substances are presented as odorants or tastants, they may undergo chemical or biological changes leading to mixtures at receptor sites. Since foods and beverages are mixtures of potential chemosensory stimulants, mixtures also occur in commercial applications. One of the most extensive commercial applications of mixtures is the use of high fructose corn syrups. These mixtures predominantly contain different levels of glucose and fructose. These sweeteners along with others containing sucrose, glucose and high intensity sweeteners are used very broadly in foods and beverages throughout the food industry.

**Synergy and Isoboles:** When two substances produce the same type of effect; such as cardiac stimulation, sweet taste or a particular odor quality; the concentrations of each compound in a mixture that produce the same level of response can be determined. An isobole is a plot of the concentrations of the substances that produce the same level of effect. In a taste or olfactory experiment, the points on one of these plots are referred to as *points of subjective equality*.

Two isoboles are shown in Figure 1. The line marked “addition” shows a linear relationship between the concentrations of A and B that produce equal effects. From the curve marked “synergy”, it can be seen that as the concentration of A increases, the corresponding amount of B decreases but not in a linear fashion. In fact, the total concentration of both substances together is less than that predicted from a linear isobole. Points of subjective equality for sweetness have been determined in humans for various concentrations of glucose and fructose. The isoboles are convex. This suggests a synergistic relationship between these sweeteners. It would be useful to provide a molecular explanation for the occurrence of synergy in humans. The challenge is to explore this hypothesis without biochemical evidence and to test molecular models using psychophysical data.

**Figure 1. Linear and non-linear isoboles.**



**Models Based on the Law of Mass Action:** When a substance (A), an agonist, binds a receptor (R) to produce an agonist-receptor complex (AR), it is assumed that this binding process is reversible,



From the Law of Mass Action

$$[AR]/[R_t] = K_a[A]/\{1+K_a[A]\}, \quad (1)$$

where [A] represents the concentration of A,  $K_a$  is the association constant for A binding to R, and  $[R_t]$  is the total receptor concentration. Under the assumption that the effect produced, such as sweet taste intensity or response to a drug, is a linear function of activated receptors, equation 1 can be used to model the fraction of the total response associated with A as a function of [A]. It should be noted that when modeling isobole data this linearity assumption is strong and unnecessary.

To extend these ideas to model sweet taste synergy, it is helpful to:

1. Replace linearity with a monotonicity assumption,
2. Allow for the possibility of a transducer entity, and
3. Include mixture effects.

The monotonicity assumption states that as [AR] increases, the measured response changes uni-directionally. This assumption allows for a broad range of dose-response functions, including the linear function. The need to introduce a transducer entity arises from research on the transduction mechanism. The transduction mechanism is a process by which a stimulus induces a potential change in a receptor cell. Compound A binds receptor R to produce complex AR. This complex interacts with transducer T resulting in the for-

mation of ART. It is now assumed that the intensity of the percept is monotonically related to [ART]. These reactions can be represented as

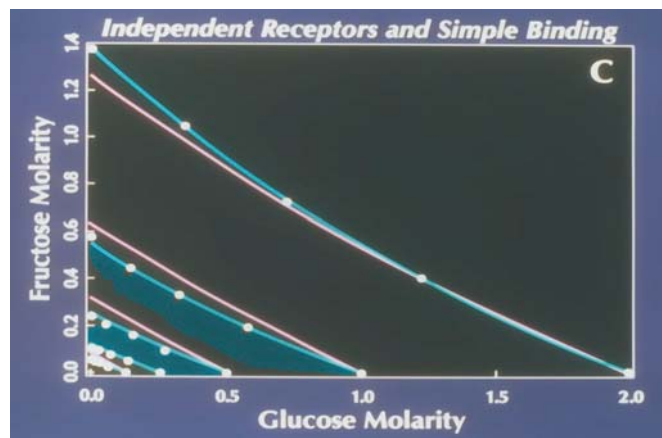


The association constant,  $K_a$ , for the initial binding to a receptor is a measure called *affinity* and the association constant,  $K_{ar}$ , for the second reaction is a measure called *efficacy*. A compound could have a high affinity and no efficacy such as a  $\beta$ -blocker or taste inhibitor. In general, a compound's effect depends on its various levels of affinity and efficacy. These ideas have been used to develop a general model<sup>1,2</sup> for the mixture effects of any number of compounds binding any number of receptors and transducers. This model explains the synergistic effects of glucose and fructose in mixtures.

**Application to Glucose/Fructose Mixtures:** De Graaf and Frijters<sup>3</sup> found the total concentration of mixtures of fructose and glucose that are as sweet as glucose alone at various target concentrations (0.125, 0.25, 0.5, 1.0 and 2.0 molar). The mixtures contained fructose at 25%, 50%, 75% and 100%. From these data it is possible to construct isoboles for glucose and fructose showing the points of subjective equality. De Graaf and Frijters used the method of constant stimuli to find the points of subjective equality. This method involves paired tests of a target concentration of glucose and particular glucose/fructose mixtures to determine the sweeter stimulus. From these data the points of subjective equality were derived.

Figure 2 shows two special cases of the general mixture model. In both cases independent systems are assumed. In one case (blue) a transducer is included and in the other (red) there is no transducer. The transducer model fits the data (the marked points) significantly better than the alternative. Although more complex special cases of the general mixture model also fit the glucose/fructose data, these cases have been discussed elsewhere<sup>4</sup> and will not be discussed here.

**Figure 2. Isobole data and two model fits.**



The isobole equation for the independent receptor-transducer model<sup>1</sup> is

$$[B_m] = \alpha \{[A] - [A_m]\} / \theta \quad (2)$$

where  $[A_m]$  and  $[B_m]$  are the concentrations of glucose and fructose in the mixture,  $[A]$  is the target concentration of glucose,  $\alpha$  and  $\theta$  are parameters that depend on the affinities and efficacies of A and B, and  $\theta$  additionally depends on  $[A]$ .

**Applications to Food and Beverage Products:** In this article, the discussion focused on models for mixture effects in simple systems. For more complex systems, such as a carbonated beverage, mixture effects of product components for one sensory attribute may depend on other attributes. The ideas discussed are a useful starting point for these applications, but may require modification to take other taste qualities into account. Central suppression of sweetness by sourness is one possible effect that may need to be considered. Mixture research on food and beverage products should determine what extensions of the mixture models need to be made.

**Implications:** Linear isoboles cannot explain synergy. Simple binding to common receptors and transducers produce linear isoboles. Independent receptor and transducer systems with simple binding will predict synergy and it can be seen from Figure 2 that this model accounts for the synergistic effect of glucose/fructose mixtures in humans.

Psychophysical data, such as that of De Graaf and Frijters<sup>3</sup>, can be used to make inferences about molecular mechanisms at the periphery. This is possible because the type of intensity matching data used only requires a weak (monotonic) assumption about the connection between events at the periphery and mental percepts. Models were then built using the Law of Mass Action that were based on peripheral binding parameters. From these parameters, inferences can be drawn about the presence of a transducer, the type of binding and the relative affinity and efficacy of different chemosensory stimulants. Examination of the fit to the mixture data shows that fructose may be sweeter than glucose because of its greater affinity though weaker efficacy. Antagonists, or blockers, have high affinity (they occupy receptors) but no efficacy. This leads to the unexpected conclusion that although fructose is sweeter than glucose in humans it may be more similar to an inhibitor of sweet taste.

#### References:

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2. Ennis, D.M. (1991). Molecular mixtures models based on competitive and non-competitive agonism. *Chemical Senses*, **16**, 1-17.
3. De Graaf, C. and Frijters, J.E.R. (1986). A psychophysical investigation of Beidler's mixture equation. *Chemical Senses*, **11**, 295-314.
4. Ennis, D.M. (1991). Modeling the sweet taste of mixtures. *Food Technology*, **45**, 140, 142, 145.