APPENDIX

Laing et al [6] produced a hypothetical model linking stromal refractive index with thickness as follows:

$$N= (n_s t_1 + n_w t_2 - n_w t_1)/t_2 \qquad \text{eq. 1}$$

The various terms are listed in Table A1.

Table 1. List defining the various terms used in the hypothetical models equating stromal refractive index with hydration. *Obtained from Maurice.[4] **Obtained from Patel et al.[25]

N= refractive index of the stroma
H= hydration of the stroma (mass of water/mass of dry matter in the stroma)
e=density of water/density of dry matter in the stroma=0.67gm/cc*
n_1= refractive index of the dry stromal collagen (=1.55)*
n_2= refractive index of the stromal interstitial fluid or ground substance
n_4=refractive index of stromal keratocytes (=1.401)**
n_s = refractive index of the stroma when thickness = t_1
n_w =refractive index of water (=1.333)
c= concentration of solutes in the stroma(=0.045gm/ml)*
R= refractive increment (=0.18ml/gm)*
x_1= volume fraction of dry collagen at normal physiological hydration.
x_2= volume fraction of stromal keratocytes
t_1 = initial thickness of stroma (mm).
t_2 = new thickness of stroma (mm)
b= is the reciprocal of the density of ground substance in the stroma and, constant in the linear stromal hydration- thickness relationship (H= at-b)

Their model was based on the assumption that, there was a direct linear decrease in central corneal thickness as water was expressed from the cornea. The opposite occurring as water was imbibed into the cornea. The linear relationship between stromal hydration (H) and thickness (t) is confirmed when hydration is defined as the mass of water present in the stroma divided by the mass of dry material present in the stroma. Eq. 1 can be modified as now follows by substituting hydration for thickness when hydration changes from H_1 to H_2:

$$N= [n_s(H_1 + b) + n_w(H_2 – H_1)]/[H_2+b] \quad \text{eq. 2}$$

The model is limited because the prediction depends heavily on two constants namely, the start values for both refractive index and hydration of the stroma. It does not allow for predicting the refractive index for other variables such as the optical properties of stromal collagen, ground substance or keratocytes. The refractive index of the stroma can be estimated using other models that were proposed for estimating the refractive index of either liquid or gaseous mixtures.[9,30] Theoretical models encapsulating various parameters are useful for not only predicting the relative influence of various individual parameters on net refractive index but also, for comparing empirical data with theoretical models to refine the power of prediction and isolate the chief source of unexpected results.

The Gladstone-Dale law of mixtures [9] was used as a starting point to estimate stromal refractive index.[4, 5, 7, 8]

Maurice [4] stated:

$$N = n_1d_1 + n_2d_2$$

Where $d_1+d_2=1$. And, assuming all organic substances are dissolved in the interstitial stromal fluid

$$n_2= n_3 + cR \quad \text{eq. 2i}$$

Maurice argued, eq. 2i may be true in vitro but, in vivo stromal collagen must be hydrated to some extent. Hence, the terms n_1 and d_1 should be modified and substituted with $(n_1 + n_2h_1)/(1+h_1)$ and $d_1(1+h)$ respectively so:

$$N= n_1 d_1+n_2h_1d_1+n_3d_2 +cRd_2 \quad \text{eq. 2ii}$$

Fatt and Harris [5] expanded on Maurice's argument and derived a numerical model by considering the definitions of d_2 and hydration. Their equation suggests there is a tendency for a close reciprocal association between N and H as follows:

$$N= 1.5581 - [0.215H/(H+e)]$$

In algebraic terms, this reads as follows:

$$N = n_1[1-(H/(H+e))] + n_2(H/(H+e))+ cR \qquad \text{eq. 7}$$

According to Maurice, the hydration of stromal collagen is a constant. Therefore, if the overall hydration of the stroma should change then the value of c, the concentration of solutes in the stroma, would change accordingly. If other substances, such as insoluble proteins, were present in the form of a suspension forming part of the total ground substance then, these substances may also have an associated specific hydration (call it Hm). By definition, H= [mass of water hydrating collagen (Wc) + mass of water diluting the solutes (W) + mass of water hydrating the suspension (Wm)] ÷ [mass of collagen (K) + mass of solutes (K1) + mass of suspension (Km)].

Thus, $H= (Wc+W+Wm)/(K+K1+Km)$ and, $c= K1/ [H(K+K1+Km)-Wc-Wm]$.

Wm is negligible in relation to Wc so it follows $c= K1/ [H(K+K1+Km)-Wc]$.

He noted, in the stroma weight for weight 76% of the fluid is equivalent to aqueous humour, 19% is collagen, 2% is insoluble, and 3% is soluble ground substance. Therefore, K+K1+Km =24%, and for a stroma of unit mass, total dry weight =0.24, K=0.19, Km=0.02, K1=0.03. He also stated that water makes up 55% of the stromal fibrils. Hence, Wc:K=55:45 and as K= 0.19, Wc=0.19x55/45.

By substituting values and simplification, c= 0.125/(H-0.9675925).

Worthington[7] and Meek et al[8] considered various likely permutations resulting in more refined models equating stromal refractive index with overall hydration. However, all of these approaches ignored the likely effects of the stromal keratocytes on the overall refractive index of the stroma. If we assume that, the hydration of the stromal collagen and keratocytes remain constant as water either leaves or enters the stroma then n_1 in eq. 7 can be replaced with a single term that combines the influences of both collagen and the keratocytes on N. Thus, $n_5 = (n_1x_1 + n_4x_2)/(x_1+x_2)$. Assuming n_5 remains constant as overall stromal hydration either rises or falls and appropriately substituting the terms of n_5 into eq. 7 results in:

$$N = \{[(n_1x_1 + n_4x_2)/(x_1+x_2)].[1-(H/(H+e))]\} + n_2(H/(H+e))+ cR \qquad \text{eq. 8}$$

The refractive index of keratocytes will also depend on the magnitude of the intrinsic hydration. We do not have a definitive value for this refractive index. However, estimates for the mean refractive index of human epithelial cells at the centre of the cornea in vivo range from 1.397 [27] to 1.401 [25]. The latter figure was used to substitute for n_4 in eq. 8 in our computations.