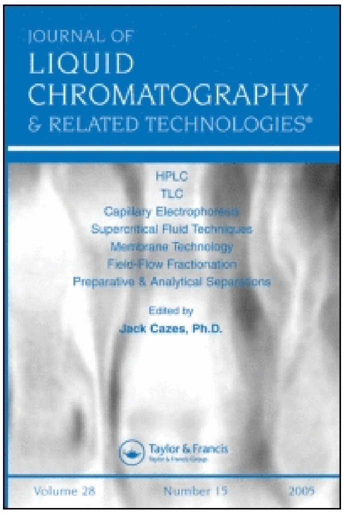

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An Analysis of Imperfect Resolution in the Chromatography of Particle Suspensions

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AN ANALYSIS OF IMPERFECT RESOLUTION IN THE CHROMATOGRAPHY OF PARTICLE SUSPENSIONS

by

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ABSTRACT

Here-in is reported a general method of correction for imperfect resolution in the chromatography of particle suspensions. It overcomes most of the limitations of previously reported methods. A non-linear particle diameter - retention volume calibration curve and a generalised spreading function are considered. Moment equations are developed for two types of general detectors; the first type includes a refractive index detector and a turbidity detector with Rayleigh scattering while the second type includes a tubidity detector with Mie scattering. The moment equations were applied to the analysis of chromatograms of narrow Dow polystyrene latices measured by size exclusion chromatography.

INTRODUCTION

Axial dispersion phenomenon is a serious imperfection in the chromatography of particle suspensions. The input sample W(y) is distorted as a result, so that the diameter frequency distribution calculated based on the measured response F(v) may be significantly in error. Considerable effort has been expended in the literature to solve the integral equation which relates F(v) to W(y)[1]. However, no single method of calculating W(y) is completely satisfactory and by and large, the numerical techniques perform poorly when the size distribution is complex. Rather,F than solve for W(y) and then the size distribution, one might try to take an easier route and solve for the moments of the distribution. This problem is fortunately more tractable and two methods of solution have recently been reported.

The first method after Hamielec and Singh[2] utilises bilateral Laplace transforms and relates true diameter averages to measured averages through simple correction factors. Their solutions, given for a general detector which includes a refractive index detector and a turbidity detector with Rayleigh scattering, accounts for skewing but is restricted to a linear particle diameter- retention volume calibration curve. More recently, Husain et al[3] presented solutions which are applicable in addition to the previous detector types to a turbidity detector with Mie scattering. A unique feature of this method is the calculation of diameter averages as a function of retention volume. The solutions were developed for a Gaussian spreading function and both linear and non-linear calibration curves.

The present analysis is a rather general one. It considers a non-linear calibration curve and a general statistical shape function proposed by Provder and Rosen[4]. The analysis permits simple correction factors to be derived for a turbidity detector with Mie scattering, hitherto not possible. Consider the following development.

THEORY

The detector response F(v) to an input sample W(y) is given by the integral equation

$$F(v) = \int_{-\infty}^{\infty} W(y)G(v-y)dy \qquad (1)$$

where G(v-y) is the instrumental spreading function. The commonly used Gaussian spreading function is inadequate when single species chromatograms are skewed. In the following analysis, we therefore consider the generalised spreading function suggested by Provder and Rosen. It is given as

$$G(v-y) = G_0(v-y)\ [1 + \sum_{n=3}^{\infty} A_n\ H_n(x)/n!] \tag{2}$$

where

$$G_0(v-y) = (1/2\pi\sigma^2)^{1/2}\ \exp[-(v-y)^2/2\sigma^2] \tag{3}$$

$$x = (v-y)/\sigma \tag{4}$$

$H_n(x)$ are the Hermite polynomials and the coefficients A_n are functions of the nth order moments, μ_n of $G(v-y)$ about y. A special case of eqn. (2) is called the Edgeworth series and is obtained when $A_6 = 10\ A_3^2$ and $A_n=0$ for $n \geq 7$. $H_n(x)$ and A_n for n upto 6 are given in Table 1. The first two coefficients are of direct statistical significance - A_3 provides an absolute statistical measure of skewness while A_4 is a measure of the flattening or kurtosis of the spreading function.

Eqn. (2) may be rewritten in the form (truncated beyond n=6)

$$G(v-y) = G_0(v-y)\ a_0(1 + \sum_{n=1}^{6} a_n(v-y)^n/\sigma^n) \tag{5}$$

TABLE 1

n^{th} Order Hermite Polynomials And Coefficients A_n

n	$H_n(x)$	A_n
3	$x^3 - 3x$	$\mu_3/\mu_2^{3/2}$
4	$x^4 - 6x^2 + 3$	$\mu_4/\mu_2^2 - 3$
5	$x^5 - 10\ x^3 + 15\ x$	$\mu_5/\mu_2^{5/2} - 10\ \mu_3/\mu_2^{3/2}$
6	$x^6 - 15\ x^4 + 45\ x^2 - 15$	$\mu_6/\mu_2^3 - 15\ \mu_4/\mu_2^2 + 30$

where

$$a_0 = 1 + A_4/8 - A_6/48 \tag{6}$$

$$a_1\, a_0 = -A_3/2 + A_5/8 \tag{7}$$

$$a_2\, a_0 = A_6/16 - A_4/4 \tag{8}$$

$$a_3\, a_0 = A_3/6 - A_5/12 \tag{9}$$

$$a_4\, a_0 = -A_6/48 + A_4/24 \tag{10}$$

$$a_5\, a_0 = A_5/120 \tag{11}$$

$$a_6\, a_0 = A_6/720 \tag{12}$$

Analytical solutions of the moment equations are now developed. The term a_0 is not carried through the analysis since it cancels out in the moment expressions. Two detector types are considered, namely,

$$\text{Type 1} \qquad F(v) \propto N(v)\, D^{\gamma}(v) \tag{13}$$

$$\text{Type 2} \qquad F(v) \propto N(v)\, D^{\gamma}(v)\, K(v) \tag{14}$$

where N(v) and K(v) are the number concentration and extinction coefficient of particles with diameter D(v). Type 1 represents both the refractive index detector (γ=3) and the turbidity detector scattering according to Rayleigh theory (γ=6). Type 2 includes the turbidity detector scattering according to Mie theory. It follows from eqns. (13) & (14) and the definition of a moment

$$M_k = \int_{-\infty}^{\infty} D^k\, f(D)\, dD \tag{15}$$

where f(D) is the normalized particle diameter frequency distribution, that

$$\text{Type 1} \quad M_k(uc) = \int_{-\infty}^{\infty} F(v)\, D(v)^{k-\gamma}\, dv \Big/ \int_{-\infty}^{\infty} F(v)\, D(v)^{-\gamma}\, dv \tag{16}$$

$$\text{Type 2} \quad M_k(uc) = \int_{-\infty}^{\infty} F(v)\, D(v)^{k-2}\, K(v)^{-1}\, dv \Big/ \int_{-\infty}^{\infty} F(v) D(v)^{-2} K(v)^{-1} dv \tag{17}$$

$M_k(c)$ are similarly defined by replacing $F(v)$ by $W(y)$ (subscripts uc and c designate the uncorrected and corrected or true respectively). Eqns. (16) and (17) are of the form

$$M_k(\ ) = \bar{M}_k(\ )/\bar{M}_0(\ ) \tag{18}$$

If $\phi_k(v)$ is defined as

Type 1 $$\phi_k(v) = D(v)^{k-\gamma} \tag{19}$$

Type 2 $$\phi_k(v) = D(v)^{k-2}\, K(v)^{-1} \tag{20}$$

then

$$\bar{M}_k(uc) = \int_{-\infty}^{\infty} F(v)\phi_k(v)\, dv \tag{21}$$

Figini[5] recently proposed the solution of an equation similar to eqn. (21). Rewriting it with the help of eqn. (1) yields

$$\bar{M}_k(uc) = \int_{-\infty}^{\infty} W(y)\, I_k(y)\, dy \tag{22}$$

where

$$I_k(y) = \int_{-\infty}^{\infty} \phi_k(v)\, G(v-y)\, dv \tag{23}$$

Consider a nonlinear particle diameter-retention volume calibration curve given by

$$\ell n\, D(v) = A' - B'v + C'v^2 \tag{24}$$

The fit of extinction coefficients $K(v)$ calculated from Mie theory is represented similarly by

$$\ell n\, K(v) = A'' - B''v + C''v^2 \tag{25}$$

It follows from eqns. (19), (20), (24) and (25) that

$$\phi_k(v) = \exp(kA - kBv + kCv^2) \qquad (26)$$

where

Detector	$k\underline{A}$	$k\underline{B}$	$k\underline{C}$	
Type 1	$(k-\gamma)A'$	$(k-\gamma)B'$	$(k-\gamma)C'$	(27)
Type 2	$(k-2)A'-A''$	$(k-2)B'-B''$	$(k-2)C'-C''$	(28)

Since in the final moment equations, A, B and C do not appear except as their product with k, the above equations are valid for all k including k=0. Let,

$$P_k = (1 - 2\,kC_\sigma^{\,2})^{-1} \qquad (29)$$

$$\alpha k = kB_\sigma^{\,2} \qquad (30)$$

$$R_k = \sigma^2 P_k[(kB)^2/2 - 2(kA)(kC)] \qquad (31)$$

then the solution of eqn. (23) is given by

$$I_k(y) = \exp(R_k)\ \phi_k^{\,P_k}(y) \sum_{n=0}^{6} Q_{n,k}\ y^n \qquad (32)$$

where the coefficients $Q_{n,k}$ are given in Table 2. The integrals required to obtain the above solution are given in the Appendix. Note that when $P_k=1$, $Q_{n,k}(n=1,...6)$ vanish and $Q_{0,k}$ simplifies to

$$Q_{0,k} = 1 + \sum_{n=3}^{n=6} (-1)^n\ P_k^n\ (\alpha_k/\sigma)^n\ A_n/n! \qquad (33)$$

The upper limit in the summation may be higher if additional terms are considered in the shape function. It follows from eqns. (18), (22) and (32) that

$$M_k(uc) = \exp(R_k - R_0)\,\frac{\sum_{n=0}^{6} [Q_{n,k} \int_{-\infty}^{\infty} y^n\, W(y)\, \phi_k^{P_k}(y)\, dy]}{\sum_{n=0}^{6} [Q_{n,0} \int_{-\infty}^{\infty} y^n\, W(y)\, \phi_0^{P_0}(y)\, dy]} \tag{34}$$

Consider the simplifications of eqn. (34) for each detector type

Type 1, Case A: kC=0, i.e., linear calibration curve, and generalized spreading function

Since

$$kC = 0 \tag{35}$$

therefore

$$P_k = 1 \tag{36}$$

$$Q_{n,k} = 0 \quad n = 1, 2 \ldots \tag{37}$$

Hence

$$M_k(c)/M_k(uc) = \exp[\sigma^2 k\,(\gamma - k/2)\, B'^2]\,Q_{0,0}/Q_{0,k} \tag{38}$$

$Q_{0,k}$ is given according to eqn. (33). For a skewed spreading function where A_3 alone is considered, this equation yields the results derived by Hamielec et al. shown in Table 3

Type 1, Case B: kC≠0, i.e., non-linear calibration curve and Gaussian spreading function

When the spreading function is Gaussian.

$$Q_{0,k} = \sqrt{P_k} \tag{39}$$

$$Q_{n,k} = 0 \quad n=1, 2 \ldots \tag{40}$$

Therefore

$$\frac{\dfrac{M(c)}{[(k-\gamma)P_k + \gamma]}}{\dfrac{M(c)}{[(1-P_0)\gamma]^k}\quad M_k(uc)} = \exp(R_0-R_k)\,\sqrt{(P_0/P_k)} \tag{41}$$

It is obvious that a direct relationship between the k^{th} corrected and uncorrected moments is not obtained.

TABLE 2

Coefficients $Q_{n,k}$ in Eqn. (32)(subscript k on   and P are omitted for convenience)

Coefficient	Expression
$Q_{0,k}$	$\sqrt{P}\ \{1-\alpha P\frac{a_1}{\sigma} + P(\sigma^2 + \alpha^2 P)\frac{a_2}{\alpha^2} - \alpha P^2(3\sigma^2 + \alpha^2 P)\frac{a_3}{\sigma^3} + P^2[\alpha^2 P\ (6\sigma^2 + \alpha^2 P) + 3\ \sigma^4]\ \frac{a_4}{\sigma^4}$
	$-\alpha P^3(\alpha^4 P^2 + 10\ \alpha^2\sigma^2 P + 15\ \alpha^4)\frac{a_5}{\sigma^5} + [P^4\alpha^2(\alpha^4 P^2 + 15\ \alpha^2\sigma^2 P + 45\ \sigma^4) + 15\ P^3\alpha^6]\ \frac{a_6}{\sigma^6}\ \}$
$Q_{1,k}$	$\sqrt{P}(P-1)\ \{\ \frac{a_1}{\sigma} - 2\alpha P\ \frac{a_2}{\sigma^2} + 3P(\sigma^2 + \alpha^2 P)\ \frac{a_3}{\sigma^3} - 4\alpha P^2(3\sigma^2 + P\alpha^2)\frac{a_4}{\sigma^4}$
	$+5P^2(3\ \sigma^4 + 6\ \sigma^2\alpha^2 P + \alpha^4 P^2)\frac{a_5}{\sigma^5} - 6\alpha P^3(15\ \sigma^4 + 10\ \sigma^2\alpha^2 P + \alpha^4 P^2)\frac{a_6}{\sigma^6}\ \}$

$Q_{2,k}$	$\sqrt{P}(P-1)^2 \{ \frac{a_2}{\sigma^2} - 3\alpha P \frac{a_3}{\sigma^3} + 6P(\sigma^2 + \alpha^2 P)\frac{a_4}{\sigma^4} - 10\alpha P^2(3\,\sigma^2 + P\alpha^2)\frac{a_5}{\sigma^5}$ $+ 15P^2[\alpha^2 P\,(6\,\sigma^2 + \alpha^2 P) + 3\,\sigma^4]\frac{a_6}{\sigma^6} \}$
$Q_{3,k}$	$\sqrt{P}(P-1)^3 \{ \frac{a_3}{\sigma^3} - 4\alpha P \frac{a_4}{\sigma^4} + 10\,P(\sigma^2 + \alpha^2 P) \frac{a_5}{\sigma^5} - 20\,\alpha P^2(3\,\sigma^2 + P\alpha^2) \frac{a_6}{\sigma^6} \}$
$Q_{4,k}$	$\sqrt{P}(P-1)^4 \{ \frac{a_4}{\sigma^4} - 5\,\alpha P \frac{a_5}{\sigma^5} + 15\,P(\sigma^2 + \alpha^2 P) \frac{a_6}{\sigma^6} \}$
$Q_{5,k}$	$\sqrt{P}(P-1)^5 \{ \frac{a_5}{\sigma^5} - 6\,\alpha P \frac{a_6}{\sigma^6} \}$
$Q_{6,k}$	$P(P-1)^6 \frac{a_6}{\sigma^6}$

TABLE 3

Axial Dispersion Correction Factors

Diameter Average		Correction Factor	
	General Detector	Turbidity Detector (=6) $\alpha=0$	Refractive Index Detector (=3) $\alpha=0$
$D_n(c)/D_n(uc)$	$\exp[(\gamma-1/2)B'^2\sigma^2]\dfrac{(1+\gamma^3\alpha B'^3)}{[1-(1-\gamma)^3\alpha B'^3]}$	$\exp(11\ B'^2\sigma^2/2)$	$\exp(5\ B'^2\sigma^2/2)$
$D_s(c)/D_s(uc)$	$\exp[(\gamma-1)B'^2\sigma^2]\ \dfrac{(1+\gamma^3\alpha B'^3)^{1/2}}{[1-(2-\gamma)^3\alpha\ B'^3]^{1/2}}$	$\exp(10\ B'^2\sigma^2/2)$	$\exp(4\ B'^2\sigma^2/2)$

$D_v(c)/D_v(uc)$	$\exp[(\gamma-3/2)B'^2\sigma^2]\frac{(1+\gamma^3\alpha\ B'^3)^{1/3}}{[1-(3-\gamma)^3\alpha\ B'^3]^{1/3}}$	$\exp(9\ B'^2\sigma^2/2)$	$\exp(3\ B'^2\sigma^2/2)$
$D_{ss}(c)/D_{ss}(uc)$	$\exp[(\gamma-5/2)B'^2\sigma^2]\frac{[1-(2-\gamma)^3\alpha\ B'^3]}{[1-(3-\gamma)^3\alpha B'^3]}$	$\exp(7\ B'^2\sigma^2/2)$	$\exp(B'^2\sigma^2/2)$
$D_w(c)/D_w(uc)$	$\exp[(\gamma-7/2)B'^2\sigma^2]\frac{[1-(3-\gamma)^3\alpha\ B'^3]}{[1-(4-\gamma)^3\alpha\ B'^3]}$	$\exp(5\ B'^2\sigma^2/2)$	$\exp(-B'^2\sigma^2/2)$
$D_t(c)/D_t(uc)$	$\exp[(\gamma-9/2)B'^2\sigma^2]\frac{[1-(3-\gamma)^3\alpha\ B'^3]^{1/3}}{[1-(6-\gamma)^3\alpha\ B'^3]^{1/3}}$	$\exp(3\ B'^2\sigma^2/2)$	$\exp(-3\ B'^2\sigma^2/2)$

Type 1, Case C: kC≠0 i.e., non-linear calibration curve, and generalised spreading function

Let

$$\overline{y_k^n} = \int_{-\infty}^{\infty} y_n\, W(y)\phi_k^{P_k}(y)\, dy \Big/ \int_{-\infty}^{\infty} W(y)\, \phi_k^{P_k}(y)\, dy \qquad (42)$$

Then

$$\frac{M(c)^{[(k-\gamma)P_k+\gamma]}}{M(c)^{[(1-P_0)\gamma]}\, M_k(uc)} = \exp(R_0-R_k)\frac{\sum_{n=0}^{6} Q_{n,0}\, \overline{y_0^n}}{\sum_{n=0}^{6} Q_{n,k}\, \overline{y_k^n}} \qquad (43)$$

Since $\overline{y_k^n}$ is defined in terms of the unknown distribution W(y), the practical use of eqn. (43) depends upon obtaining a suitable approximation for $\overline{y_k^n}$. This will be discussed later.

Type 2, Case A: kC=0 i.e., linear calibration curve, linear extinction coefficient fit, and generalized spreading function

Eqns. (35)-(37) are applicable and hence

$$M_k(c)/M_k(uc) = \exp[\sigma^2 kB'(-kB'/2+2B'+B'')]Q_{0,0}/Q_{0,k} \qquad (44)$$

It is significant to note that as in the case of the other detectors, a simple correction factor has been derived for the turbidity detector in the Mie scattering regime. A linear calibration curve is not uncommon in chromatography. The condition of a linear extinction coefficient fit is more difficult to satisfy; however if the spreading function is not excessively broad, this condition may be approached. Relationships for some important diameter averages are given in Table 4. Note that when B"=4B' (Rayleigh scattering), the formulae for the diameter averages reduce to those derived by Hamielec et al (Table 3)

TABLE 4

Axial Dispersion Correction Factors for the Turbidity Detector in the Mie Scattering Region (Linear Calibration Curve, Linear Extinction Coefficient Fit and Generalized Spreading Function)

Diamter Average	Correction Factor*
$D_n(c)/D_n(uc)$	$\exp[\sigma^2 B'(3B'/2+B'')]Q_{0,0}/Q_{0,1}$
$D_s(c)/D_s(uc)$	$\exp[\sigma^2 B'(B' + B'')][Q_{0,0}/Q_{0,2}]^{1/2}$
$D_v(c)/D_v(uc)$	$\exp[\sigma^2 B'(B'/2+B'')][Q_{0,0}/Q_{0,3}]^{1/3}$
$D_{ss}(c)/D_{ss}(uc)$	$\exp[\sigma^2 B'(-B'/2+B'')]\ Q_{0,2}/Q_{0,3}$
$D_w(c)/D_w(uc)$	$\exp[\sigma^2 B'(-3B'/2+B'')]\ Q_{0,3}/Q_{0,4}$
$D_t(c)/D_t(uc)$	$\exp[\sigma^2 B'(-5B'/2+B'')][Q_{0,3}/Q_{0,6}]^{1/3}$

*$Q_{0,k}$ $1+\sum_{n=3}(-1)^n(\alpha_k/\sigma)^n A_n/n!$ (upper summation limit not specified)

Type 2, Case B: $kC \neq 0$ and Gaussian spreading function

Eqns. (39) and (40) are applicable and hence,

$$M_k(uc) = \exp(R_k-R_0)\,\sqrt{(P_k/P_0)}\int_{-\infty}^{\infty} W(y)\,\phi_k^{P_k}(y)\,dy \Big/ \int_{-\infty}^{\infty} W(y)\,\phi_0^{P_0}(y)\,dy \qquad (45)$$

Let

$$P_k = 1 + f_k \qquad (46)$$

Therefore

$$\int_{-\infty}^{\infty} W(y)\,\phi_k^{P_k}(y)\,dy \equiv \int_{-\infty}^{\infty} W(y)\,\phi_k(y)\,\phi_k^{f_k}(y)\,dy \qquad (47)$$

If $\phi_k^{f_k}(y)$ is approximated as

$$\phi_k^{f_k}(y) = [D(y)^{k-2}\,K^{-1}(y)]^{f_k} \simeq \text{constant}\,[D(y)^{k-2}\,D(y)^{-\beta}]^{f_k} \qquad (48)$$

where the extinction coefficient is assumed proportional to $D^{\beta}(y)$, then

$$\int_{-\infty}^{\infty} W(y)\,\phi_k^{P_k}(y)\,dy \simeq \text{constant}\ \overline{M}_{(k+g_k)}(c) \qquad (49)$$

where

$$g_k = (k-2-\beta)f_k \qquad (50)$$

The choice of β must be made contingent on the value of the constant in eqn. (49) being independent of k. This allows eqn. (45) to be simplified to

$$M_k(uc) = \exp(R_k-R_0)\sqrt{P_k/P_0}\ \overline{M}_{(k+g_k)}(c)\,/\,\overline{M}_{g_0}(c)$$

$$= \exp(R_k-R_0)\sqrt{P_k/P_0}\ M_{(k+g_k)}(c)\,/\,M_{g_0}(c)$$

or alternately

$$\frac{M(c)_{(k+g_k)}}{M(c)_{g_0}\ M_k(uc)} = \exp(R_0-R_k)\sqrt{P_0/P_k} \tag{51}$$

To apply this equation, $k+g_k$ is set successively equal to 0,1,2 ... and solved for k. Using the corresponding uncorrected moments, the ratios $M_0(c)/M_{g_0}(c)$, $M_1(c)/M_{g_0}(c)$, $M_2(c)/M_{g_0}(c)$ etc. can be calculated and hence $M_1(c)$, $M_2(c)$ etc. since $M_{0_0}(c)$ ■ 1

Compared to the previously reported method[3], this method is apparently simpler. However, it lacks the attractive feature of the previous method namely, a calculation of the size variation across the chromatogram. When Rayleigh scattering theory is applicable, β is identically equal to 4, $B''=4B'$, $f_k = g_k=0$ and eqn. (51) yields the formulae derived by Hamielec et al. Note that when Rayleigh scattering theory is not applicable, β may still be approximated as 4; however, now $B''\neq 4B'$, $f_k\neq 0$, $g_k\neq 0$.

Type 2, Case C: kC≠0 and generalised spreading function

In accordance with the development in Case B, one obtains

$$\frac{M(c)_{(k+g_k)}}{M(c)_{g_0}\ M_k(uc)} = \exp(R_0-R_k)\ \frac{\sum_{n=0}^{6} Q_{n,0}\ \overline{y_0^n}}{\sum_{n=0}^{6} Q_{n,k}\ \overline{y_k^n}} \tag{52}$$

It is seen from the preceding analysis, that it is only when kC=0 that a direct relationship exists between $M_k(c)$ and $M_k(uc)$; otherwise $M_k(uc)$ is related to a ratio of true moments. In terms of application, this presents however, no difficulty.

Calculation of $\overline{y_k^n}$ and choice of β

When W(y) is narrow, it is reasonable to approximate $\overline{y_k^n}$ by v_p^n where v_p is the peak retention volume. This is shown later for the experimental chromatograms of narrow particle standards. It is conceivable that when W(y) is broad and axial dispersion relatively small, $\overline{y_k^n}$ may be calculated by replacing W(y) in eqn. (42) by the actual chromatogram. Alternately $\overline{y_k^n}$ may be set equal to $\bar{v}_n$ where $\bar{v}$ is the mean retention volume based on the actual chromatogram.

If Rayleigh scattering theory is applicable then the value of β would be identically equal to 4. Otherwise it is < 4 and varies with the particle size range. A value in the range $2<\beta<4$ is proposed.

The above suggestions were evaluated using synthesized chromatograms for a turbidity detector in the Mie scattering regime [eqns. (51) and (52)]. Extinction coefficients were adequately fitted by the function, eqn. (25). It was more convenient to compare uncorrected diameter averages with those calculated directly from F(v). The results are shown in Tables 5 and 6. The calculations of the diameter averages for a specific value of β with and without the approximation $\overline{y_k^n} \simeq \bar{v}^n$, were almost identical and are not shown. It is observed that calculations based on the moment equation for $\beta=2$, 3 and 4 do not differ significantly from each other and agree closely with those calculated from F(v) directly. The assumption of $P_k = 1$ [eqns. (51) and (52) reduce to eqn. (44)] leads to significantly erroneous results even though the actual value of P_k is only slightly different from unity. In other case studies made where a P_k value as high as 1.2 was used, the above approximations were found to be equally valid.

Application of some of the equations derived in this section are now demonstrated for experimental chromatograms of narrow distribution polystyrene latices. It is appropriate to mention here that though the spreading function parameters were considered

TABLE 5

Evaluation of the Moment Equations [Eqns. (51) and (52)] for the Type 2 Detector

Calibration curve : $A' = 8.0158$ $B' = 0.1707$ $C' = 0$
Extinction coefficient fit : $A'' = 1.2674$ $B'' = -0.2042$ $C'' = -0.0166$
$W(y)$: Gaussian with mean retention volume 20 and variance 2
P_k : 1.071 (independent of k)

$G(v-y)$:	Gaussian , $\sigma^2 = 2.0$						$\sigma^2 = 2.0$; $a_1 = -0.4$; $a_i = 0$, $i=2,\ldots 6$					
Diameter Average	D_n	D_s	D_v	D_{ss}	D_w	D_t	D_n	D_s	D_v	D_{ss}	D_w	D_t
<u>Uncorrected</u>												
1. From F(v)	56.7	60.6	64.8	74.1	84.7	96.8	69.5	73.1	77.2	85.9	96.4	108.6
2. From moment equation ($\beta=2$)	57.0	61.0	65.3	74.7	85.3	97.4	68.5	72.3	76.4	85.4	96.0	108.4
3. From moment equation ($\beta=3$)	56.8	60.7	65.0	74.3	84.9	97.0	68.2	71.9	76.0	85.0	95.6	107.9
4. From moment equation ($\beta=4$)	56.5	60.4	64.6	74.0	84.5	96.5	67.9	71.6	75.7	84.6	95.1	107.4
5. From moment equation assuming $P_k=1$	75.4	82.7	82.5	96.1	108.4	122.1	83.3	91.0	90.4	104.4	117.0	131.2
<u>Correct or True</u>												
6. From W(y)	76.8	79.2	81.8	87.1	92.6	98.5	76.8	73.1	77.2	85.9	96.4	108.6

TABLE 6

Evaluation of Moment Equations [Eqns. (51) and (52)] for the Type 2 Detector

Calibration curve : $A' = 8.0158$ $B' = 0.1707$ $C' = 0$
Extinction coefficient fit : $A'' = 1.2674$ $B'' = -0.2042$ $C'' = -0.0166$
$G(v-y)$: $\sigma^2 = 2.0$; $a_1 = -0.4$; $a_i = 0$, $i=2 \ldots 6$
P_k : 1.071 (independent of k)

W(y) :	Lognormal with parameters $\ln 20$ and 0.01						2 Peak W(y) composed of 2 Gaussian peaks having means 18.0 and 22.0 ; variance, 2.0					
Diameter Average	D_n	D_s	D_v	D_{ss}	D_w	D_t	D_n	D_s	D_v	D_{ss}	D_w	D_t
<u>Uncorrected</u>												
1. From F(v)	65.5	69.6	74.1	83.8	94.8	107.1	64.7	67.1	70.4	77.5	89.9	108.0

2.	From moment equation (β=2)	64.2	68.5	73.0	83.1	94.4	106.9	63.4	65.9	69.2	76.4	88.9	107.1
3.	From moment equation (β=3)	63.7	68.0	72.6	82.7	94.0	106.4	63.3	65.7	69.0	76.0	88.2	106.2
4.	From moment equation (β=4)	63.3	67.6	72.2	82.2	93.5	105.9	63.1	65.5	68.7	75.6	87.5	105.3
5.	From moment equation assuming P_k=1	78.8	87.5	86.3	102.0	115.2	129.5	77.1	82.9	82.7	94.5	109.2	129.6
	Corrected or True												
6.	From W(y)	72.6	75.7	78.7	85.0	91.2	97.2	71.0	72.5	74.5	78.8	86.4	97.3

independent of retention volume in deriving solutions to the integral equation, in practice they are slowly varying functions. When applying these equations to experimental chromatograms, however, error resulting from use of constant average parameter values is not likely to be significant.

APPLICATION TO EXPERIMENTAL CHROMATOGRAMS

Fig. 1 shows the chromatograms for the 85, 109, 176 and 220 nm polystyrene latices. The calibration data and measured variances are shown in Table 7.

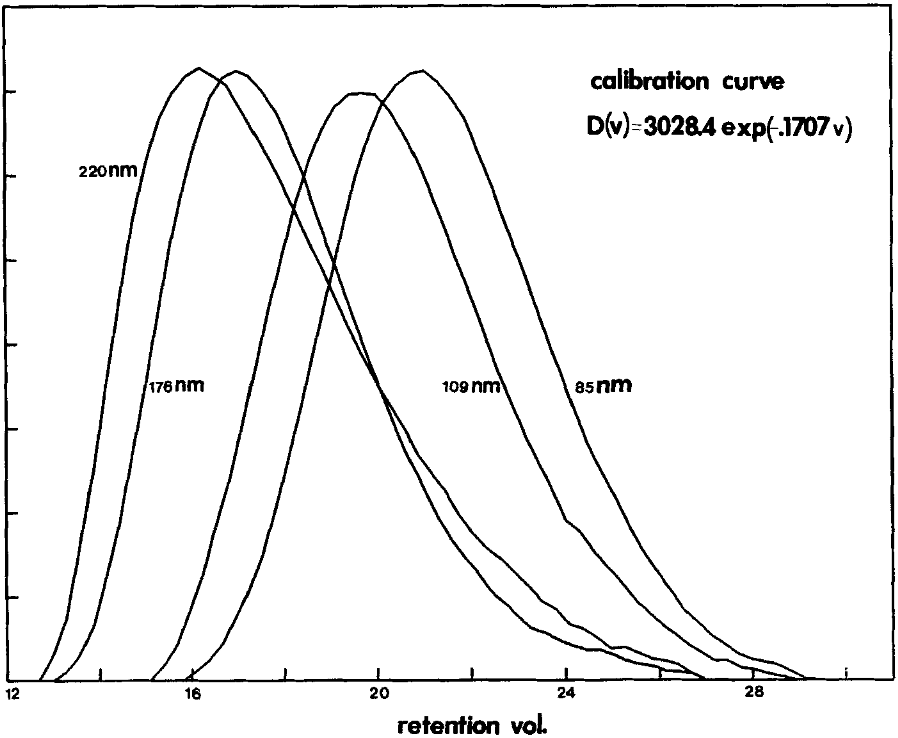

Figure 1: Chromatograms of narrow distribution polystyrene latices.

TABLE 7

Calibration Data and Measured Variances

Sample	85	109	176	220
Peak Retention Volume ($m\ell$)	21.02	19.70	17.00	15.35
Measured Variance ($m\ell^2$)	5.15	5.59	5.37	3.58

The uncorrected diameter averages are given in Table 8. For the smaller diameter latices calculations based on Mie and Rayleigh theories, particularly for the lower diameter averages do not differ considerably from each other. Observe that axial dispersion corrections would have to be very significant for these data. Application of Rayleigh correction factors is now shown.

TABLE 8

Uncorrected Diameter Averages Calculated from Rayleigh and Mie Theories

Row 1 Calculated from Rayleigh theory

Row 2 Calculated from Mie theory

Sample	D_n	D_s	D_v	D_{ss}	D_w	D_t
85	33.2	35.1	37.5	42.8	50.6	60.1
	34.1	36.5	39.5	46.3	56.5	68.4
109	35.7	38.0	41.0	47.5	57.6	70.2
	37.3	40.3	44.3	53.6	67.8	83.8
176	46.4	49.8	54.5	65.2	83.0	104.0
	51.2	57.1	65.0	84.4	111.4	135.0
220	89.3	94.1	100.0	112.8	130.3	149.9
	104.9	113.1	122.3	143.2	166.3	186.3

A. Application of Rayleigh Correction Factors

The relationship between the corrected and the uncorrected moment is given by

$$M_k(c)/M_k(uc) = \exp[\sigma^2 k(6 - k/2)\, B'^2]\; Q_{0,0}/Q_{0,k} \tag{38a}$$

Considering only the coefficients A_3 and A_4 and setting

$$\varepsilon_1 = (B'\,\sigma)^3\, A_3/6 \tag{53}$$

$$\varepsilon_2 = (B'\,\sigma)^4\, A_4/24 \tag{54}$$

the following relationships can be derived.

$$D_n(c) = D_n(uc)\, \exp(11\, \sigma^2\, B'^2/2)\, \frac{(1+216\, \varepsilon_1 + 1296\, \varepsilon_2)}{(1+125\, \varepsilon_1 + 625\, \varepsilon_2)} \tag{55}$$

$$\frac{[D_n(c)/D_n(uc)]^{10/11}}{D_s(c)/D_s(uc)} = \left(\frac{1+216\varepsilon_1 + 1296\varepsilon_2}{1+125\varepsilon_1 + 625\varepsilon_2}\right)^{10/11} \left(\frac{1+64\varepsilon_1 + 256\varepsilon_2}{1+216\varepsilon_1 + 1296\varepsilon_2}\right)^{1/2} \tag{56}$$

$$\frac{[D_n(c)/D_n(uc)]^{9/11}}{D_v(c)/D_v(uc)} = \left(\frac{1+216\varepsilon_1 + 1296\varepsilon_2}{1+125\varepsilon_1 + 625\varepsilon_2}\right)^{9/11} \left(\frac{1+27\varepsilon_1 + 81\varepsilon_2}{1+216\varepsilon_1 + 1296\varepsilon_2}\right)^{1/3} \tag{57}$$

If both A_3 and A_4 are non-zero then eqns. (56) and (57) are solved for ε_1 and ε_2 using a non-linear algebraic equations solver and σ^2 is estimated from eqn. (55). If A_4 is considered zero then only eqns. (55) and (56) need be solved. Calculation results are shown in Table 9. It is obvious from these results that the parameter A_3 and A_4 are significant; the significance of A_5 and A_6 could not be tested due to a difficulty in solving the additional equations. However, the calculated results with the two shape parameters are quite satisfactory. An alternate scheme to calculate σ^2 and A_n from the moment equations is developed later and does not have the present difficulty.

TABLE 9

Diameter Averages of Latices Measured at 254 nm - Application of Rayleigh Correction Factors

Sample	σ^2	A_3	A_4	D_n	D_s	D_v	D_{ss}	D_w	D_t
85	3.67	1.944	0.	85.2	84.7	83.9	82.3	79.1	77.1
	5.12	1.812	-2.134	85.2	86.4	87.1	88.5	86.7	82.2
109	4.51	1.919	0.	108.7	108.1	107.1	105.2	100.5	95.8
	5.90	1.896	-1.975	108.7	109.7	110.3	111.7	109.3	101.8
176	5.75	1.603	0.	175.6	173.8	172.6	170.3	164.4	152.9
	6.87	1.719	-1.467	175.6	175.2	175.9	177.2	175.5	160.6
220	3.13	3.629	0.	220.2	221.6	221.5	221.2	209.5	194.6
	4.15	3.277	-3.429	220.2	223.2	225.3	229.5	222.3	203.4

B. Application of Mie Correction Factors

The pertinent equation to be solved is

$$\frac{M(c)_{(k+g_k)}}{M_{g_0}(c)\ M_k(uc)} = \exp(R_0-R_k)\ \frac{\sum_{n=0}^{6} Q_{n,0}\ \bar{y}_o^n}{\sum_{n=0}^{6} Q_{n,k}\ \bar{y}_k^n} \qquad (52)$$

Consider now how each of the terms in the above equation is evaluated. Two cases are treated. In the first case, the parameters of the spreading function were obtained by the numerical recovery of G(v-y) from eqn. (1) using the frequency distribution data of the latices[6] as measured by electron microscopy. In the second case, the parameters were considered as variables as in the application of Rayleigh correction factors and their values were obtained by solving eqn. (52).

TABLE 10

Estimation of $\overline{y_k^n}$ (the range corresponds to k=0 to 24)

	85 nm	109 nm	176 nm	220 nm
n=1				
Eqn.42	20.9-20.6	19.5-19.4	16.7-16.6	15.4-15.3
v_p^n	21.0	19.7	17.0	15.4
n=6				
Eqn.42	$8.52\text{-}7.66\times10^7$	$5.50\text{-}5.29\times10^7$	$2.16\text{-}2.13\times10^7$	$1.32\text{-}1.28\times10^7$
v_p^n	8.63×10^7	5.85×10^7	2.41×10^7	1.31×10^7

It was earlier stated that when W(y) is narrow, $\overline{y_k^n}$ may be approximated by v_p^n where v_p is the peak elution volume. Table 10 compares $\overline{y_k^n}$ calculated rigorously according to eqn. (42) (using electron microscopy frequency data) with its approximation v_p^n. Excellent agreement is observed. $\overline{y_k^n}$ is essentially independent of k.

As previously discussed the choice of β must be made contingent on the value of the constant in eqn. (49) being independent of k. Table 11 indicates that β values in the range 0.75-4.25 are adequate. The calculations were done using the electron microscopy frequency data.

TABLE 11

Estimation of the Value of the Constant in Eqn. (49)
(Range Corresponds to k=0 to 6)

	85 nm	109 nm	176 nm	220 nm
β=0.75	2.83- 2.80	2.89- 2.88	2.27- 2.27	1.61- 1.61
β=4.25	68.90-69.50	124.4 -124.7	112.1 -112.2	20.6 - 20.6

TABLE 12

Diameter Averages of Latices Measured at 254 nm - Application of Mie Correction Factors (Row 1 $\beta=2$, Row 2 $\beta=3$, Row 3 $\beta=4$)

Sample	D_n	D_s	D_v	D_{ss}	D_w	D_t
	86.2	83.7	81.9	78.4	77.2	77.0
85	85.0	82.7	81.1	78.0	77.0	76.9
	83.8	81.8	80.4	77.7	76.9	76.9
	108.6	104.2	101.1	95.2	93.1	92.6
109	106.2	102.3	99.6	94.5	92.9	92.5
	104.1	100.7	98.4	94.0	92.7	92.4

The Edgeworth series provides among the four samples, the best representation of the spreading function (obtained by solving eqn. (1)) for the 85 and 109 nm samples. The results of applying eqn. (52) to these two samples are shown in Table 12. As expected, the calculations are insensitive to the value of β chosen. For $\beta=2$, the number average diameter for both samples are remarkably on target; however the higher diameter averages are slightly lower. *Note that these calculations are based on* parameters which have been estimated independently of the moment equations; no atempt has been made to force the data fit unlike in the case of the application of Rayleigh correction factors.

Two probable causes of error in the analysis are 1) the Edgeworth series does not adequately describe the tail ends of the spreading function. Consideration of coefficients A_n, n>6 when $kC_3 \neq 0$, is impractical due to the complexity in deriving the coefficients $Q_{n,k}$. 2) Error results from the use of $+\infty$ and $-\infty$ integration limits in the analysis when in reality a species of mean retention volume y is dispersed between finite retention volume limits. The effect of this was investigated on the solution of eqn. (23). The ratio

$$\varepsilon = \int_{-\infty}^{\infty} D^{k-2}(v)\ K^{-1}(v)\ G(v-y)\ dv \Big/ \int_{a}^{b} D^{k-2}(v)\ K^{-1}(v)\ G(v-y)\ dv \qquad (58)$$

is calculated where a and b are the actual retention volume limits. The results are shown in Table 13. It is evident that significant error occurs for k=0 and 1 and that beyond k=6, the results become rapidly non-sensical.

It is likely that a good estimation of the lower diameter averages results from the mutually compensating effects of errors 1 and 2. For the higher diameter averages, error 1 alone is the probable cause.

Consider now the second case where the parameters of the spreading functions are calculated directly from the moment equations. The summation terms in eqn. (52) are linear in the coefficients A_n. If therefore a value of σ^2 is assumed, the term

$$\frac{M_{(k+g_k)}(c)\quad \exp(R_k-R_0)}{M_{g_0}(c)\quad M_k(uc)}$$

TABLE 13

Estimation of ε [Eqn. (58)]

k	85 nm sample a=15.8 b=30.0 y=21.7	109 nm sample a=15.1 b=29.2 y=20.7	176 nm sample a=13.0 b=27.0 y=18.3	220 nm sample a=12.8 b=23.2 y=16.4
0	0.69	0.54	0.51	0.83
1	0.85	0.75	0.71	0.92
2	0.94	0.89	0.88	0.97
3	0.98	0.97	0.96	0.99
4	0.99	0.99	0.99	1.00
5	1.00	1.00	1.00	1.01
6	1.00	1.00	1.00	1.02
10	0.45	0.47	0.06	0.25
15	$4.7.10^{-4}$	$4.7.10^{-5}$	$1.5.10^{-5}$	$5.6.10^{-4}$

can be evaluated and hence A_n obtained by solving a set of simultaneous linear equations. Convergence occurs when the sum of squares, of the deviations of the calculated diameter averages from their known true values, attains a minimum. When A_3 alone is considered, only one equation is solved using a value of k corresponding to $k+g_k=0$. Thereafter each additional parameter requires additional equations corresponding ~~to~~ $k+g_k = 1, 2 \ldots$ etc. The calculation results are shown in Table 14 for all four samples indicating excellent agreement with actual diameter averages of the samples. Note that the parameter values calculated differ significantly from the actual variance, skewness and kurtosis (bracketted quantities in Table 14) obtained by the numerical recovery of G(v-y) from eqn. (1).

DISCUSSION

In the preceding analysis, extinction coefficients based either on Rayleigh or Mie theory were used even though they differ from the actual measured coefficients[7]. The discrepancy between the measured extinction coefficients and those based on theory will therefore be reflected in the values of the spreading function parameters. The purpose of the analysis was to demonstrate the capability of the theory to predict with a good degree of accuracy, the diameter averages from the chromatograms of narrow distribution latices. Since calculated extinction coefficients display the same trend as the measured ones, it is immaterial whether the actual coefficients were used or not. It might be tempting therefore to conclude that the simpler Rayleigh correction factors be always used in preference to the more complex Mie correction factors. However, in a practical situation, the purpose of analysing narrow distribution standards is to yield spreading function parameters to be used as calibration constants for the analysis of unknown polydisperse samples. The success of their size measurement will depend in general on whether or not the theoretical values of the extinction coefficients agree with experimental values.

TABLE 14

Diameter Averages of Latices Measured at 254 nm – Application of Mie Correction Factors ($\beta=2$)

Sample	2	A_3	A_4	A_5	A_6	D_n	D_s	D_v	D_{ss}	D_w	D_t
85	3.2	4.72	0	0	0	86.4	87.1	86.9	86.3	82.5	83.4
	4.2	2.50	-2.15	0	0	85.2	85.2	84.6	83.6	81.1	82.0
	5.4	1.84	-3.51	2.12	0	85.2	85.4	85.2	84.9	83.0	83.9
	8.5 (5.13)	0.44 (0.396)	-2.53 (-0.099)	4.16	-2.47	85.2	85.4	85.5	85.9	84.9	85.1
	4.0	4.99	0	0	0	111.0	112.0	112.0	112.0	105.0	108.0

109	5.0	2.61	-1.96	0	0	109.0	108.0	107.0	105.0	101.0	103.0
	6.7 (5.62)	1.76 (0.509)	-3.34 (-0.0137)	2.0	0	109.0	109.0	108.0	107.0	103.0	108.0
176	5.2	3.86	0	0	0	180.0	181.0	178.0	173.0	160.0	186.0
	6.0 (5.34)	2.53 (0.737)	-1.43 (0.483)	0	0	176.0	175.0	172.0	166.0	157.0	181.0
220	2.7	6.61	0	0	0	221.0	218.0	212.0	200.0	194.0	228.0
	5.1 (3.58)	1.70 (0.716)	-2.21 (0.093)	0	0	220.0	217.0	212.0	202.0	194.0	220.0

Bracketted numbers represent actual variance, skewness and kurtosis.

The range of rigorous validity of Rayleigh theory is very small. Heller[8] assessed its more important range of practical validity using Mie data as a reference. His results are shown in Table 15 where Δ_a, the percent deviation is defined as

$$\Delta_a = 100(a_R - a_M)/a_R \tag{59}$$

where $a = \pi D/\lambda$. λ is the wavelength of light in the medium.

Table 15 indicates that when m ~ 1.26 (the value used for polystyrene latices in water at 254 nm), Rayleigh theory under-estimates the particle diameter by more than 10% for a ($=\pi D/\lambda$) equal to 1.2. This implies that for particles larger than ~ 80 nm error in excess of 10% could be expected for a turbidity measurement at 254 nm. However, comparison of the uncorrected diameter averages in Table 8 indicate that the upper diameter limit corresponding to > 10% error may be much higher depending upon the average diameter considered. In any given situation, it would be extremely useful to map out a region well

TABLE 15

Percent deviation Δ_a

	$a=\pi D/\lambda$					
m*	0.2	0.4	0.6	0.8	1.0	1.2
1.00	-0.55	-2.1	-4.6	-8.0	-11.9	...
1.05	-0.40	-1.8	-4.1	-7.2	-10.9	...
1.10	-0.40	-1.6	-3.6	-6.4	-9.9	...
1.15	-0.35	-1.3	-3.0	-5.6	-8.9	-13.0
1.20	-0.25	-1.1	-2.5	-4.9	-7.9	-12.0
1.25	-0.20	-0.8	-2.0	-4.0	-6.9	-11.0
1.30	-0.15	-0.6	-1.5	-3.2	-5.8	-10.0
1.33	-0.15	-0.5	-1.4	-2.85	-5.2	-9.4

*m ratio of refractive index of particle to the refractive index of the medium beyond the rigorous validity of Rayleigh theory, where errors resulting from its application are acceptable.

In general, latices are expected to be 'unclean' and also errors in tubidity measurement may exist. If a large number of narrow distribution standards are available, then their extinction coefficients may be measured to span the complete range of diameters. However, in a situation where the number of standards are limited, Mie theory may be applied to search for an imaginary part of the complex refractive index of the particle which best describes the measured data[9]. This value can then be used to calculate the extinction coefficients over the desired diameter range.

CONCLUSIONS

A very general analytical solution to the integral equation has been derived which enables the diameter averages obtained from the chromatographic response of a variety of detectors to be suitably corrected for axial dispersion. The theoretical treatment developed here-in should find extensive application in all forms of particle chromatography - size exclusion chromatography, hydrodynamic chromatography, capillary chromatography and field flow fractionation.

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NOMENCLATURE

D	Particle diameter
D_n	Number average diameter [$= M_1$]
D_s	Surface average diameter [$= M_2^{1/2}$]
D_{ss}	Specific surface average diameter [$= M_3/M_2$]
D_t	Turbidity average diameter [$= (M_6/M_3)^{1/3}$]
D_v	Volume average diameter [$= M_3^{1/3}$]
D_w	Weight average diameter [$= M_4/M_3$]
$f(D)$	Normalized particle diameter frequency distribution function
$F(v)$	Measured chromatogram
G	Instrumental spreading function
K	Extinction coefficient
M_k	kth moment of $f(D)$
N	Number concentration of particles
v, y	Retention volume
$W(y)$	True chromatogram

APPENDIX

Solution of Equation (23)

Consider the solution of the integral

$$J_a = \int_{-\infty}^{\infty} \exp(-p\,x^2 + 2qx)\, x^a\, dx \qquad (A.1)$$

Let

$$x = \frac{q}{p} + \frac{y}{\sqrt{p}} \qquad (A.2)$$

Then

$$J_a = \frac{\exp(q^2/p)}{\surd\overline{p}} \int_{-\infty}^{\infty} \exp(-y^2)\left(\frac{q}{p} + \frac{y}{\surd\overline{p}}\right)^a dy$$

$$= \frac{\exp(q^2/p)}{\surd\overline{p}} \int_{0}^{\infty} \exp(-y^2)\left(\frac{q}{p} + \frac{y}{\surd\overline{p}}\right)^a dy +$$

$$\int_{0}^{\infty} \exp(-y^2)\left(\frac{q}{p} - \frac{y}{\surd\overline{p}}\right)^a dy \qquad (A.3)$$

To solve the integrals in A.3 we require the following integral

$$I = \int_{0}^{\infty} x^m \exp(-b\, x^2)dx = \frac{\Gamma[(m+1)/2]}{2b^{(m+1)/2}} \qquad (A.4)$$

where the gamma function in the numerator is defined as

$$\Gamma(\tfrac{1}{2}) = \surd\overline{\pi} \qquad (A.5)$$

$$\Gamma(m+\tfrac{1}{2}) = \frac{1.3.5....(2m-1)}{2^m}\surd\overline{\pi} \qquad m=1,2,3\ ... \qquad (A.6)$$

The solutions of equation (A.3) for a=1 ... 6 are given by

$$J_1 = \surd(\frac{\pi}{p})\ \exp(\frac{q^2}{p})\ \frac{q}{p} \qquad (A.7)$$

$$J_2 = \surd(\frac{\pi}{p})\ \exp(\frac{q^2}{p})[(\frac{q}{p})^2 + \frac{1}{2p}] \qquad (A.8)$$

$$J_3 = \surd(\frac{\pi}{p})\ \exp(\frac{q^2}{p})[(\frac{q}{p})^3 + \frac{3}{2}\frac{q}{p^2}] \qquad (A.9)$$

$$J_4 = \surd(\frac{\pi}{p})\ \exp(\frac{q^3}{p})[(\frac{q}{p})^4 + 3\frac{q^2}{p^3} + \frac{3}{4}\frac{1}{p^2}] \qquad (A.10)$$

$$J_5 = \sqrt{(\frac{\pi}{p})}\ \exp(\frac{q^2}{p})[(\frac{q}{p})^5 + 5\frac{q^3}{p^4} + \frac{15}{4}\frac{q}{p^3}] \qquad (A.11)$$

$$J_6 = \sqrt{(\frac{\pi}{p})}\ \exp(\frac{q^2}{p})[(\frac{q}{p})^6 + \frac{15}{2}\frac{q^4}{p^5} + \frac{45}{4}\frac{q^2}{p^4} + \frac{15}{8}\frac{1}{p^3}] \qquad (A.12)$$