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CHOICE OF ANALOG ELECTRONIC LOW-PASS FILTERS IN CHROMATOGRAPHY

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SUMMARY

Butterworth low-pass filters, frequently used to improve signal-to-noise ratios in chromatographic instrumentation, are chosen for their frequency domain characteristics; their time domain response may introduce spurious peaks which are eliminated by use of the Bessel filter which provides improved chromatographic response.

INTRODUCTION

Electronic filtering is used in many analytical instruments to improve the signal-to-noise ratio. Due to the increased demands placed on detector sensitivity, requirements for electronic filtering are becoming more apparent. Low-pass filters enhance identification and quantitation of peaks at low-signal levels in both gas and liquid chromatography and in practice both analog and digital filters are used. Although digital filters allow the greatest flexibility[1,2], they usually require microprocessor control and thus many instruments still utilize analog systems due to their simplicity in implementation. Of the many types of low-pass filters, the Butterworth filter is usually chosen for its frequency domain characteristic, *i.e.*, maximally flat response with increasing frequency and rapid attenuation asbove the cut off frequency. One major limitation of this filter is its time domain characteristic of considerable overshoot for a signal with a rise time less than that of the filter. We have observed that this characteristic induces large errors of measurement of chromatographic peak areas, especially when an automatic signal integrator is used. In an attempt to reduce measurement errors, we have examined the time domain characteristic of other low-pass filters, notably the Bessel filter.

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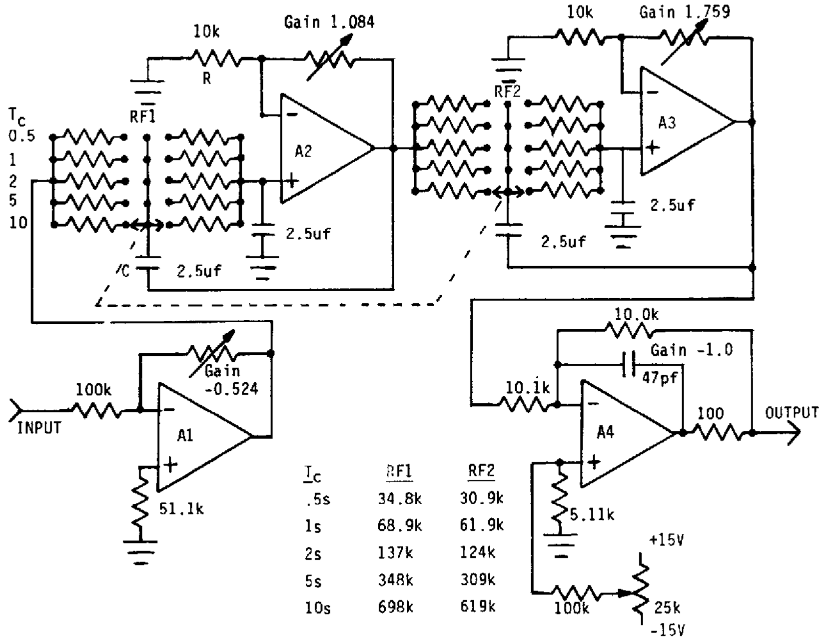
EXPERIMENTAL

Electronic

Three- and four-pole active filters were constructed following the guidelines in the *Active Filter Cookbook*[3]. The filter (Fig. 1) is designed to operate with nominally 0 to 10 V signals. Amplifier A1 reduces the input signal to compensate for the gain of the active filter amplifiers A2 and A3, and amplifier A4 acts as an output buffer. The active filter section was designed using first and second order equal-component-value Sallen–Key low-pass sections[3]. The filters were designed to have response times (0 to 90%) of between 0.5 and 10 s, depending upon selection of resistors RF1 and RF2 (Fig. 1). The damping factors, which specify the type of filter (*i.e.*, Bessel, compromise or Butterworth), were set by adjusting the gain values according to tables of ref. 3 (pp. 74–76). The values in Fig. 1 are for a four-pole Bessel (Thompson) filter. To obtain Butterworth and compromise (Paynter or transitional Thompson–Butterworth) filters, the resistors RF1 and RF2 were changed proportional to the values of Table I and the gain (gain = $3-d$ where d is the damping coefficient) was adjusted. To obtain the three-pole filter, the first filter section (amplifier A2) was changed to a first order filter by removing one bank of RF1 resistors, the resistor labelled R and the capacitor C. The gain of the resulting amplifier is unity as no damping factor is defined. To measure the time domain response a step signal 0 to 4.0 V or pulse signal (rise time less than 5 ms) was applied to the filter (on the 10 s



T_c	RF1	RF2
.5s	34.8k	30.9k
1s	68.9k	61.9k
2s	137k	124k
5s	348k	309k
10s	698k	619k

Fig. 1. Fourth order Bessel filter based on the equal-component Sallen–Key filter sections. It may be converted to a three-pole filter by removal of R, C and one section of RF1 (see ref. 3 for details). A1–A4 are OP-07 precision operational amplifiers. Capacitors are polyester and resistors are 1% metal film. The ±15 V supplies power to each operational amplifier and are decoupled with 10 ohm resistors and 0.05 μf disc capacitors. T_c = Response time (0 to 90%).

TABLE I

VALUES USED FOR DESIGN OF NOMINAL 10-s RESPONSE TIME ACTIVE FILTERS

k = kΩ; m = MΩ.

Type	*Calculated* T_c *(s)*	*Actual Tc (s)*	*RF1*	*RF2*	*Gain of A1*	*Gain of A2*	*Gain of A3*
Three-pole filters							
Bessel	7.3	8.0	750 k	681 k	−0.644	1.0	1.553
Compromise	–	8.3	868 k	825 k	−0.557	1.0	1.797
Butterworth*	8.3	8.7	1.00 m	1.00 m	−0.500	1.0	2.000
Four-pole filters							
Bessel**	8.1	9	698 k	619 k	−0.524	1.084	1.759
Compromise	–	9.7	825 k	787 k	−0.436	1.119	2.051
Butterworth	9.9	10.8	1.00 m	1.00 m	−0.388	1.152	2.235

* Filter design used as reference.

** Filter design in final configuration. −3 dB frequency is 0.064 Hz for all designs. Gain of first order sections is 1.0 due to voltage follower configuration. For second order sections gain = $3-d$ where d is the damping factor (ref. 3). Gain of A4 is −1.0. Calculated response time (T_c: 0 to 90%) was taken from figures of Zverev[6] and adjusted for resistor and capacitor values. Actual T_c was measured using 2.5 μf capacitors. Resistors RF1 and RF2 were scaled for R_c of 0.5, 1, 2, 5 and 10 s as in Fig. 1.

time constant setting) and the output recorded on a pen recorder (Fisher Omniscribe, rise time 0.3 s) (Fig. 2, Table II). To compare the effects of electronic filters on chromatography, three-pole Butterworth and four-pole Bessel filters were constructed and carefully adjusted so that their overall gains were within 1% of 1.00 (for a 4.0-V input step signal). The filters were used with the liquid and gas chromatographic systems below.

Liquid chromatography

A standard mixture containing 2 ng each of noradrenaline (NE), 3,4-dihydroxyphenylacetic acid (DOPAC), dopamine (DA), 5-hydroxyindoleacetic acid (5-HIAA), isoproterenol (ISP), homovanillic acid (HVA), 3-methoxytyramine (3-MT) and 5-hydroxytryptamine (5-HT) were separated and determined simultaneously by HPLC with electrochemical detection. Separations were performed at ambient room temperature on a 250 × 4.6 mm I.D. Ultrasphere I.P. analytical column packed with C_{18}, spherical 5-μm particles (Beckman, Toronto, Canada). A 15 × 3.2 mm I.D. Brownlee MPLC guard column packed with C_{18}, spherical 7-μm particles was installed between the Rheodyne M7125 injector and analytical column. A 20-μl sample holding loop was used in the injector. The mobile phase consisting of 75 m*M* monobasic sodium phosphate, 1 m*M* sodium octyl sulfate, 50 m*M* EDTA and 13% acetonitrile was adjusted to pH 2.75 with phosphoric acid, vacuum degassed before use and pumped through the column at 1.0 ml/min using a Waters M45 solvent delivery system (Millipore, Mississauga, Canada). Prior to the addition of the acetonitrile and phosphoric acid, the buffer was filtered through a 0.22 μm Millipore cellulose acetate filter. Initial experiments were performed with a coulometric detector (Coulochem 5100A, ESA, Bedford, MA, U.S.A.). In the experiment reported here the detector consisted of a BAS Model TL-5A thin-layer amperometric electrode (Mandel, Rockwood, Canada) controlled at 0.75 V *versus* a Ag/AgCl reference electrode using a potentiostat built in our laboratory. Signals from the detector were integrated using

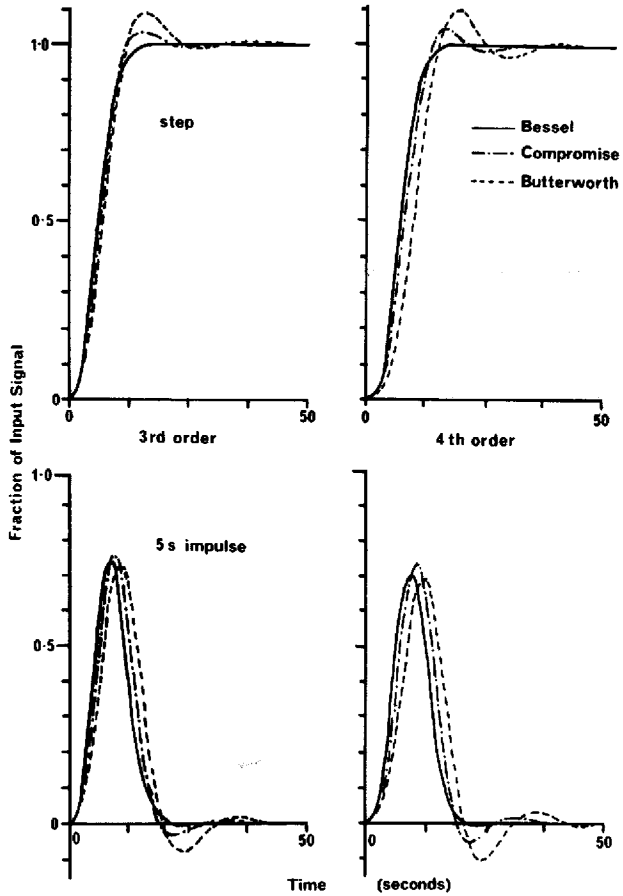


Fig. 2. Responses of third and fourth order filters to a step signal and to a 5-s wide pulse signal. Filter rise times were set to 10 s.

TABLE II

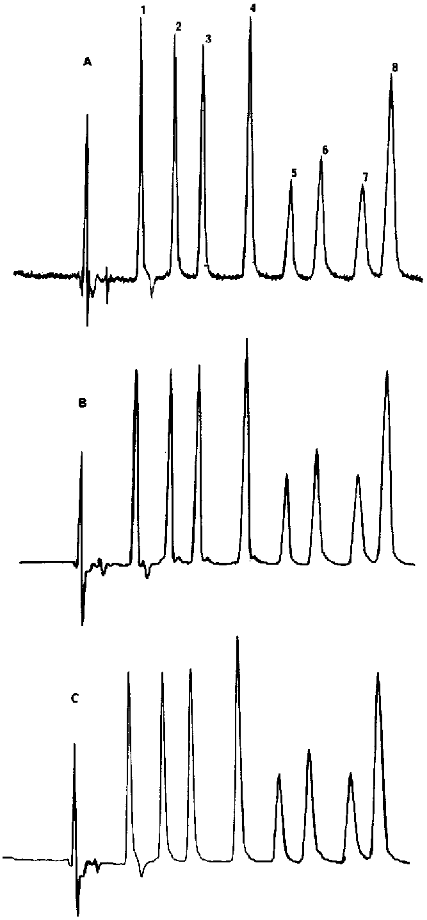
STEP RESPONSE OF ANALOG FILTERS

Type	*Theoretical** *overshoot*	*Observed*** *overshoot*
Three-pole Bessel	0	<0.6
Four-pole Bessel	0	<1.0
Three-pole Compromise	—***	3.7
Four-pole Compromise	—	5
Three-pole Butterworth	8.2	8.5
Four-pole Butterworth	11	11

* Overshoot was measured from figures from Zverev[6], pp. 402–407 as a percentage of the input step signal.

** As measured from pen chart at nominal 10-s filter time constant (see Fig. 2).

*** This type was not discussed by Zverev[6].



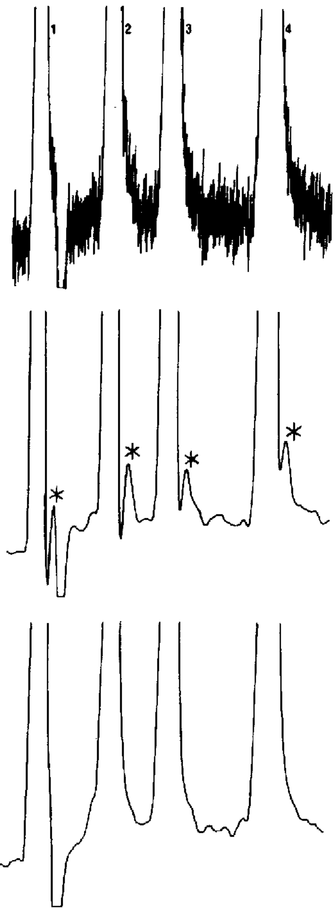


Fig. 3. Effect of filters on chromatography of catecholamines: (A) unfiltered, (B) third order Butterworth, and (C) fourth order Bessel filters at 10-s rise times. Peak identities are: 1 = NE; 2 = DOPAC; 3 = DA; 4 = 5-HIAA; 5 = ISP; 6 = HVA; 7 = 3-MT; 8 = 5-HT.

Fig. 4. Baseline sections of peaks 1 to 4: (A) unfiltered, (B) three-pole Butterworth, and (C) four-pole Bessel. Spurious peaks are marked with an asterisk.

a Spectra Physics 4290 computing integrator (Technical Marketing Associates, Calgary, Canada). Hydraulic damping of the HPLC pump was achieved by a series of volumes and constrictions; a Waters pulse damper, an empty 250 × 4.6 mm column, and a liquid filled pressure gauge each separated by 0.009 mm I.D. stainless-steel tube. Chromatograms of the 2-ng standards are shown in Figs. 3 and 4. The effect of filtering on picogram amounts of the amines is reported in Table IV.

Gas chromatography

A solution on *n*-octacosane (10 μg/ml in hexane) was injected into a Hewlett-Packard 5710 gas chromatograph which was equipped with a 2 m × 2 mm I.D. packed column containing 3% SP2100 on Supelcoport 80/100 and a flame ionization detector. The carrier gas was nitrogen at 30 ml/min. By operating the gas chromato-

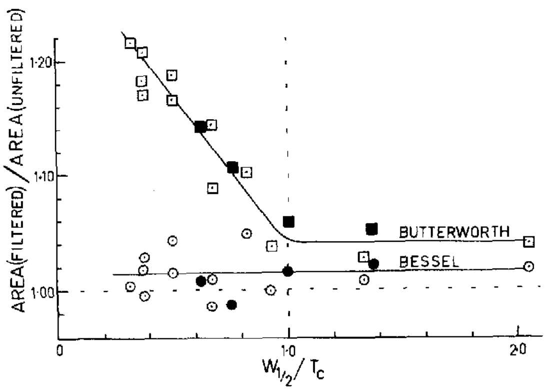


Fig. 5. Effect of filtering on gaussian peaks. Change in relative peak areas produced by four-pole Bessel and three-pole Butterworth filters with changes in peak width ($W^{1/2}$) expressed as a fraction of filter time constant (T_c). Open symbols measured with 10-s time constant; filled symbols 5-s time constant.

graph isothermally at temperatures ranging from 240 to 310°C, gaussian shaped peaks could be obtained with various peak widths. The output was recorded simultaneously on three channels using Omniscribe recorders; the raw signal, the four-pole Bessel filtered signal, and the three-pole Butterworth filtered signal at 10, 5 or 2 s time constant. The peak areas were measured from the chromatographic charts with a planimeter. The change in relative areas is shown in Fig. 5.

RESULTS AND DISCUSSION

During the development of a procedure to detect catecholmines in brain perfusate at the low picogram level using coulometric detection, it became apparent that the background noise level had to be reduced using both hydraulic pulse damping and elerctronic filtering. We observed, however, that, when the coulometric detector (ESA Coulochem) was operated using its highest time constant filter (10 s), narrow chromatographic peaks were followed by ripple, *i.e.*, a dip below baseline (which we have called undershoot) and another small peak (Figs. 3 and 4). This is due to damped oscillation of the filter to rapid rise signals[4] and obviously interfered with quantitation. We were content to operate the system with skewed peaks, produced by the long time constant of the filter as their shape was reproducible and standards enabled calibration, but the ripple characteristic was not acceptable as this changed the peak areas drastically and prevented correct operation of peak integration. Examination of the filter circuit in the Coulochem detector showed it to be a three-pole Butterworth filter, which, in another paper on high-performance liquid chromatographic (HPLC) detector improvements[5], had been extolled for its frequency domain characteristic, *i.e.*, rapid attenuation of high-frequency noise signals. The time domain character was not discussed, but the example in that paper[5] also showed some undershoot and ripple. We have examined two other analog filter types, Bessel–Thompson and compromise Thompson–Butterworth, with the aim of improving the time domain characteristic without severe loss of frequency response. The numbers of poles were chosen for their ease of construction (both require two operational amplifiers) and frequency characteristic (*i.e.*, ultimate attenuation of −18 dB for three-pole and −24 dB per octave for four-pole filters). The number of poles can be easily changed from three to four by insertion of a small number of components. Although a second order filter only requires one operational amplifier, it was not considered because of its poor cut off (−12 dB/octave) and fifth and higher orders were eliminated because of their complexity, which becomes apparent when designing switching for variable frequency units. From the Bode plots[3,6] it would appear that if the three-pole butterworth is the starting point for suitable frequency response, the four-pole Bessel, even with its slightly poorer initial roll-off, would be a good second choice.

The step and pulse responses, Fig. 2, and overshoot characteristics in Tables II and III demonstrate the superior time domain character of the Bessel filter. Theoretically the Bessel filter has no overshoot. We did find in practice that our Bessel filter had a slight overshoot of less than 1% (Table II). This was perhaps due to component selection or circuit layout. It could be reduced to zero by increasing the damping of the second filter amplifier (A3, Fig. 1) from 1.241 to about 1.324 by reducing its gain and increasing the gain of the input amplifier so that the overall gain remained at 1.00. The rise time of the four-pole Bessel (9 s) is very close to that of the three-pole Butterworth (8.7 s) at the nominal 10 s setting.

The five second square wave impulse produced a significant ripple peak (undershoot) with both the compromise and Butterworth filters. The peak areas produced by the 5 s impulse were measured planimetrically from the chart recordings, from the initial rise to the lowest point of the undershoot, *i.e.*, the same positions that the integrator selected, and compared to the area produced on the chart by the square wave. The Bessel filter was observed to produce a peak with area identical to that of the square wave, but the compromise and especially the Butterworth filters produced peaks with increased areas. In the case of the three-pole Butterworth as used in the commercial instruments, the increase in area amounted to a 19% error.

Although a compromise filter was constructed in an attempt to obtain the best

TABLE III

IMPULSE RESPONSE OF ANALOG FILTERS

Type	*Theoretical* undershoot*	*Observed undershoot***		*Relative*** area*
		2-s Pulse width	*5-s Pulse width*	
Three-pole Bessel	0.9	<0.6	<0.6	100
Four-pole Bessel	0.9	<1.0	<0.8	100
Three-pole Compromise	–[§]	2.2	4	108
Four-pole Compromise	–	3.5	6	112
Three-pole Butterworth	4.9	4.5	8	119
Four-pole Butterworth	6.7	5.8	11	124

* Measured from figures from Zverev[6] as percentages of input pulse height.

** As measured from pen chart at nominal 10-s filter time constant (see Fig. 2).

*** Area of peak produced by a 5-s pulse as a percentage of the area produced directly by the pulse.

§ This type was not discussed by Zverev[6].

characteristics of both Bessel and Butterworth filters, we decided that its overshoot response was still too large (Table II). Consequently chromatography was performed with just two types of filter, the original three-pole Butterworth and the four-pole Bessel (Fig. 3). Attenuation of peak height and the amount of distortion due to skewing was almost identical between the two filter types; in the HPLC analysis the decrease in peak height ranged from 31% for peak 1 to 5% for peak 8 (Fig. 3). The baseline response, Fig. 4, shows that the spurious peaks introduced by the three-pole Butterworth filter were not present when the four-pole Bessel was used and that the noise was attenuated by both filters.

The effect of filtering on the limit of detection of the catecholamines and metabolites was tested using the coulometric detector, Table IV. The Bessel and Butterworth filters removed the noise signal which was due primarily to fluctuations in the flow-rate caused by the pump (even after considerable hydraulic damping) with equal efficiency. At these very low levels (10 pg of each compound injected) distortion of the peak shapes was not produced by the Butterworth filter. The solvent and injection induced peaks did, however, show much greater over- and undershoot with the Butterworth filter and when larger amounts were injected, *i.e.*, 100 pg or 2 ng, distortion of the early peaks reappeared (Fig. 3). Thus the amount of overshoot is dependent upon the rate of rise of the chromatographic peaks.

In order to characterize further the effects of filtering on chromatographic peaks, the Bessel and Butterworth filters were applied to gaussian chromatographic peaks produced by *n*-octacosane injected into the gas chromatograph. The Bessel filter produced peaks with areas essentially equal to thjose of the unfiltered (raw) peaks (relative peak area: 1.014 ± 0.004, $n = 15$, mean ± S.E.M., *i.e.* 1.4% error). The area remained constant at all peak widths as seen in Fig. 5. The Butterworth filter produced peaks with areas slightly greater than those of the raw peak shapes. When the peak width (measured as width at half peak height ($W^{1/2}$), which is related to the rise time) was greater than the time constant, the peak area was about 4% larger (relative peak area: 1.042 ± 0.006, $n = 5$, mean ± S.E.M.). At peak widths less than the time constant, the error rose very rapidly to up to 20% (Fig. 5), a value similar to the error observed with the square impulse signal. When a solution containing a homologous series of hydrocarbons from parafin wax was injected, we observed very large errors (*e.g.*, up to 60%) produced in the Butterworth filtered chromatogram as the ripple peak from each chromatographic peak coalesced with the following peak, even though the two peaks were well separated in the raw and Bessel filtered chromatograms. From these results we can see that in chromatographic situations the Butterworth filter can only be used provided that the peak width is maintained greater than the filter time constant. Using HPLC under isocratic conditions, this might be difficult to achieve as the early eluting peaks may be narrower than the time constant desired to reduce the noise signal. The Bessel filter on the other hand appears to be suitable for use with peaks with widths considerably less than the filter time constant and to produce areas comensurate with the amount of compound.

During this work, two other types of analog filters were considered. A switched capacitor filter (National Semiconductor MF10) was used to construct a four-pole Butterworth filter. Although the time constant or cut-off frequency is dependent only upon the clock frequency, and is easily changed, we found the device was not designed

TABLE IV

EFFECT OF FILTERING ON DETECTION LIMITS OF CATECHOLAMINES

Compound	*Signal-to-noise ratio**		
	Unfiltered signal	*Four-pole Bessel*	*Three-pole Butterworth*
NE	2.6	22	23
DOPAC	2.0	13	14
DA	1.8	13	14
5-HIAA	1.2	10	10
ISP	1.2	8	9
HVA	0.8	7	7
3-MT	0.8	6	6
5-HT	1.0	8	8

* Signal-to-noise ratios measured using 10 pg of each compound and coulometric detector. Filters were set to 10-s time constant.

to operate at the low frequencies required for chromatography and that a large offset error and clock pulse feed through developed when we attempted to use it at frequencies below 2 Hz. To produce a Bessel filter two frequencies would be required with this unit and this reduces its convenience due to increased circuit complexity. The tapped analog delay line filter reported by Betty and Horlick[7] was also considered. It is capable of performing Savitsky–Golay type filter functions[1,2] and is thus extremely versatile. Again the minimum clock rate is anticipated to be much faster (2 kHz) than that required for our chromatographic system and we decided not to construct this type of filter.

CONCLUSION

The advantages and limitations of a number of electronic filters suitable for chromatographic use have been described and it is illustrated that the four-pole Bessel filter provides the optimal characteristics of both frequency and time domain. Little or no overshoot and undershoot is experienced with this filter and the peak areas are proportional to the amount of compound. With the three-pole Butterworth filter, however, both overshoot and undershoot are apparent and cause errors with subsequent integration, especially whjen the peak width is less than the filter time constant. Flexible higher order Bessel filters are easily constructed using the equal component Sallen–Key circuit as shown.

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REFERENCES

1 A. Savitsky and M. J. E. Golay, *Anal. Chem.*, 36 (1964) 1627–1637.
2 J. Steiner, Y. Termonia and J. Deltour, *Anal. Chem.*, 44 (1972) 1906–1909.
3 D. Lancaster, *Active Filter Cookbook*, Howard W. Sams, Indianapolis, IN, 1979.
4 L. P. Huelsman and P. E. Allen, *Introduction to the Theory and Design of Active Filters*, McGraw-Hill, Toronto, 1980.
5 R. E. Shoup, *Current Separations*, 5 (1983) 7–10.
6 A. J. Zverev, *Handbook of Filter Synthesis*, Wiley, Toronto, 1967.
7 K. R. Betty and G. Horlick, *Anal. Chem.*, 48 (1976) 2248–2253.