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Article

Gran Method for End Point Anticipation in Monosegmented Flow Titration

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Este trabalho descreve o uso do método de linearização de Gran em titulações em fluxo monossegmentado com detecção potenciométrica. O programa de controle pode estimar o ponto final após a adição de três ou quatro alíquotas de titulante. Alternativamente, o ponto final da titulação pode ser determinado pelo método da segunda derivada. Neste caso, alíquotas adicionais de titulante são adicionadas até a proximidade do ponto final e três pontos antes e após o ponto estequiométrico são usados para o cálculo de ponto final. O desempenho do sistema foi avaliado pela determinação de cloreto em soluções isotônicas e parenterais. O sistema emprega um eletrodo indicador tubular de Ag_2S/AgCl. Uma titulação típica, realizada de acordo com a definição IUPAC, requer apenas 60 μL de amostra e aproximadamente o mesmo volume de titulante ($AgNO_3$). Uma titulação completa pode ser realizada entre 1 e 5 min. Foram obtidas exatidão e precisão (desvio padrão relativo de 10 replicatas) de 2% e 1% para o método de Gran e 1% e 0,5% para o método de Gran associado ao método da segunda derivada, respectivamente. O sistema proposto reduz o tempo para realizar uma titulação, com baixo consumo de amostra e reagente, além de possibilitar uma amostragem automática completa e adição de titulante sem a necessidade de uma etapa de calibração.

An automatic potentiometric monosegmented flow titration procedure based on Gran linearisation approach has been developed. The controlling program can estimate the end point of the titration after the addition of three or four aliquots of titrant. Alternatively, the end point can be determined by the second derivative procedure. In this case, additional volumes of titrant are added until the vicinity of the end point and three points before and after the stoichiometric point are used for end point calculation. The performance of the system was assessed by the determination of chloride in isotonic beverages and parenteral solutions. The system employs a tubular Ag_2S/AgCl indicator electrode. A typical titration, performed according to the IUPAC definition, requires only 60 μL of sample and about the same volume of titrant ($AgNO_3$) solution. A complete titration can be carried out in 1 - 5 min. The accuracy and precision (relative standard deviation of ten replicates) are 2% and 1% for the Gran and 1% and 0.5% for the Gran/derivative end point determination procedures, respectively. The proposed system reduces the time to perform a titration, ensuring low sample and reagent consumption, and full automatic sampling and titrant addition in a calibration-free titration protocol.

Keywords: monosegmented flow analysis, Gran method, chloride titration

Introduction

Titration is a very simple and reliable technique, which has been widely used in different fields, such as food, chemical and pharmaceutical analyses. Its main drawback relies on the tedious and time-consuming procedure that can impair the precision and accuracy of the results if a high number of samples need to be analysed. In order to overcome this drawback, titration procedures have frequently been automated or mechanised by employing flow analysis systems. Different approaches have been proposed to implement titration in flow analysis, based on the ratio of the flow rates of the sample and titrant,[1,2] triangle-programmed flow,[3] dispersion of the sample in the titrant carrier fluid,[4] flow-batch[5] and monosegmented systems.[6-8]

Although flow titration can be performed easier and faster than manual titration, it is still slow when compared with ordinary flow methodologies, because the procedures are usually based on the dispersion concept and a calibration

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step is necessary. Therefore, many attempts have been made in order to improve sample throughput in flow titration methodologies, by employing different strategies to determine their end points.[5,7,9,10] A binary sampling algorithm has been used to determine the end point of spectrophotometric and potentiometric titrations, in which the volumetric ratio between sample and titrant solutions is varied according to a successive approximation strategy until the end point is reached. Korn *et al.*[9] have used this concept to perform acid-base spectrophotometric titration in a non-segmented flow system, while Martelli *et al.*[10] have described an acid-base potentiometric titration in a monosegmented flow system. The Fibonacci optimisation algorithm has also been employed to locate the end point of acid-base titrations carried out in monosegmented flow[7] and flow-batch hybrid systems.[5] A continuous on-line titration system has been recently proposed by Tanaka *et al.*[11] based on feedback-controlled flow ratiometry and the principle of compensating errors, and applied to neutralisation titrations. The Gran method has been also employed to determine the end point of precipitating titrations, in which mixtures of decreasing sample and increasing titrant volumes are sequentially introduced into a mixing chamber.[12]

Recently, Aquino *et al.*[13] have proposed a monosegmented flow titration system, capable of performing a true titration, according to the IUPAC definition. In this system, a sample monosegment is introduced into an air carrier stream and titrant aliquots are sequentially added to the same sample until the end point is reached. The system is controlled by a microcomputer and the sample monosegment is pumped forward to the detector and backward to the titrant addition point, as the titration is carried out. A successive approximation algorithm is employed to locate the end point, attained after, at most, eight steps, providing a precision better than 2%.

The present work describes the use of the above mentioned flow titrator to implement the Gran linearisation approach in order to estimate the end point of titration, which can, if necessary, be more accurately determined by the second derivative procedure. The feasibility of the proposed system has been evaluated with the determination of chloride in isotonic fluids and parenteral solutions by precipitating titration with standard silver nitrate solution.

Experimental

Reagents and solutions

Reagents of analytical grade were always used and solutions were prepared with distilled and deionised water. A 0.1000 mol L^{-1} silver nitrate stock solution was prepared by proper weighing and dilution of the salt and stored in a bottle protected from ambient light. More diluted solutions were prepared by proper dilution of the stock solution. A 1.0 x 10^{-3} mol L^{-1} sodium chloride solution was prepared by dilution from a 0.1000 mol L^{-1} standard solution. A 10% (m/v) potassium nitrate solution was employed to wash the system after a titration had been performed. Parenteral solutions and electrolytic beverages were purchased in local market.

Flow manifold

A flow manifold similar to that previously described[13] was adapted to incorporate a potentiometric detection system, as shown in Figure 1. A sliding bar injector[14] was used to introduce the sample monosegment into the flow system. An Ismatec IPC-4 peristaltic pump, controlled by the computer through a RS232 serial interface, was used to move the sample monosegment forward and backward. 0.8 mm i.d. PTFE tubing was used as the reaction coil. The addition of titrant to the sample was carried out by employing a home-made syringe pump,[13] furnished with a 2.5 mL gas tight glass syringe (Hamilton TLL). A Ag_2S/AgCl flow-through electrode was prepared as described elsewhere[15] and used as indicator electrode. A double junction AgCl reference electrode, filled with KNO_3 external solution, was placed downstream as close as possible to the indicator electrode. Figure 2 depicts the detection cell, where the contact between the reference electrode and carrier stream was made by means of a saturated KNO_3 salt bridge, containing 3.0% (m/v) agar-agar. The agar-KNO_3 salt bridge avoids the leaking of the KNO_3 solution and the entrapment of air bubbles at the reference electrode connection. Two optical sensors[16] placed along the PTFE tubing were employed to indicate

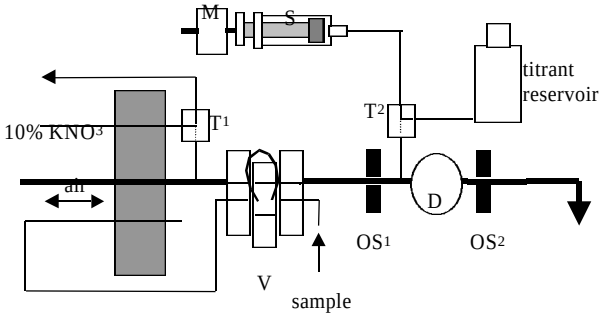

Figure 1. Manifold of the monosegmented flow titrator. P, peristaltic pump; T_{1-2}, miniature electromechanical 3-way valves; M, linear actuator with step motor; S, gas-tight syringe (2.5 mL); V, titrand injection port; OS_{1-2}, optical switches; D, potentiometric detection system. Typical air flow rate = 2.2 mL min^{-1}.

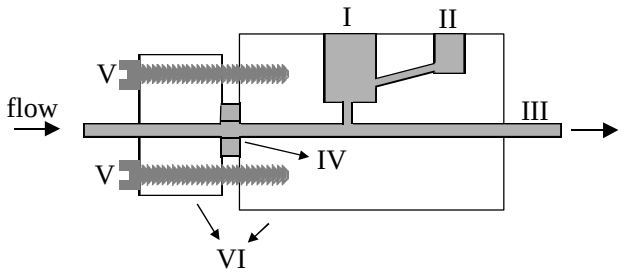

Figure 2. Details of the potentiometric flow cell. (I) compartment for the reference electrode; (II) compartment for the KNO_3 solution; (III) reaction tubing; (IV) Ag_2S/AgCl tubular indicator electrode; (V) screws for connecting acrylic blocks; (VI) acrylic blocks.

to the computer the position of the sample monosegment in order to halt the flow for titrant addition or potential measurement. The optical sensors were placed 15 cm from each other and the sample was maintained between them during the titration.

Control of the system and titration procedure

The flow titrator system was controlled by a microcomputer through an electronic parallel interface (Advantech PCL-711S), except for the peristaltic pump whose control was done through a standard RS-232 serial interface. Software, written in Microsoft VisualBasic 3.0, allowed the computer to control the system in order to carry out a complete titration, estimating its end point using the Gran method and adding titrant aliquots before and after the stoichiometric point for second derivative end point calculation.

Before starting the titration, the program was fed with the titrant aliquot volume and number of aliquots used for the Gran calculations. The titration was carried out by introducing a sample monosegment of 60 μL into the flow system, being carried by air at a flow rate of 2.2 mL min^{-1}. As the sample reached the syringe, the flow was stopped and an aliquot of titrant was added to the sample. Then, the monosegment was pumped to the flow cell and the electrical potential difference between the electrodes was measured. As defined by the user, the measurements of volume of titrant added *versus* potential were taken consecutively, by pumping the monosegment backward for titrant addition and forward for potential reading. After these measurements, the end point of the titration was calculated by employing a Gran linearisation method.[17] If a more accurate localisation of the end point is required by the user, the software considers the end point obtained by Gran method as an estimative and the titration is continued. Thus, titrant is added to the monosegment until 90% of the Gran estimated end point volume is reached and the respective electrical potential is read. The software calculates the volume of the titrant aliquots necessary to reach 110% of the estimated end point volume with five additions. Then, these five titrant aliquots are added to the monosegment, each one followed by solution homogenisation and reading of the respective electrical potential. These pairs of data are then used to calculate the end point of the titration by the second derivative method.

Results and Discussion

Precipitating titration in flow systems has to be performed in controlled conditions as the tubing can be clogged by precipitate, which can also be adsorbed by the flow-through electrode membrane. Therefore, for argentimetric determinations in flow systems the maximum concentration permitted for the analyte is about 10^{-2} mol L^{-1}. The proposed system has an additional problem to be overcome, as the sample monosegment has to be pumped forward and backward through the system for performing a titration, which contributes toward absorption of the precipitate onto the walls of the tubing and the membrane. Although it would be expected to happen, no difficulty was found during these titrations, as AgCl precipitate particles do not adhere to PTFE tubing. As a consequence, after a titration has been performed, the system was easily and completely cleaned by sequentially inserting three monosegments of a 10% KNO_3 solution.

The Gran method for determination of the end point was first evaluated by titrating a 1.0 x 10^{-3} mol L^{-1} NaCl solution with a 1.00 x 10^{-3} mol L^{-1} $AgNO_3$ standard solution. Table 1 shows the results obtained when three and four additions of titrant were employed for Gran end point calculation in the titration of 60 μL of the sodium chloride solution. As can be seen, the precision (calculated as the relative standard deviation of 10 replicates) and the accuracy do not vary significantly when either three 10.0 μL aliquots or four 7.3 μL aliquots of titrant were employed, totalling 50% of the end point volume, for the calculation of the end point. Although the accuracy was not changed to the same value, a slightly better precision

Table 1. Calculation of the end point by the Gran method. Titration of 60.0 μL of a NaCl solution with a 1.00 x 10^{-3} mol L^{-1} $AgNO_3$ standard solution (average ± standard deviation of ten replicates)

Strategy	Aliquots x Volume (μL)	Gran (x 10^{-3} mol L^{-1})	rsd (%)	Error (%)
1	3 x 10	1.174 ± 0.044	3.7	+ 1.0
2	4 x 7.3	1.179 ± 0.044	3.7	+ 1.4
3	3 x 15	1.177 ± 0.028	2.3	+ 1.3

Manual method: (1.163 ± 0.007) x 10^{-3} mol Cl^- L^{-1}.

was obtained when three titrant aliquots of 15.0 μL (totalling 75% of the expected end point volume) were added for end point calculation. This result is explained considering the performance of the syringe pump employed, which can add aliquots of titrant with a precision of 0.5 μL. Figure 3 shows the Gran plot and the second derivative curve for determination of the end point of a titration of 60.0 μL of NaCl solution with $AgNO_3$ standard solution and Table 2 shows the results obtained when the same titrations as in Table 1 continued until the second derivative end point calculation. In these cases, titrant, from the last point for Gran calculation, was continuously added up to 90% of the estimated end point, totalling ca 54.0 μL of titrant. Then, five titrant aliquots of 2.4 μL were added, and the respective potentials were measured, totalling 66 μL (110% of the end point volume). As can be noted, the precision and the accuracy obtained show an opposite behaviour, indicating there is no significant differences among these results, which vary due to experimental errors.

In order to evaluate the performance of the system and, in particular, of the syringe for addition of aliquots of titrant, the determination of the end point by the second derivative

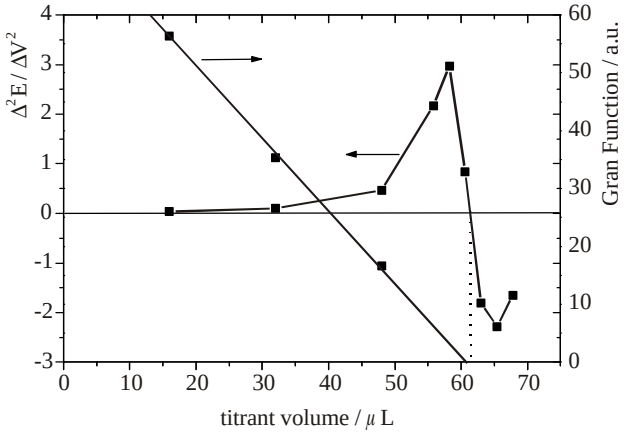

Figure 3. Gran and second derivative plots for the titration of a 1.022 x 10^{-3} mol L^{-1} NaCl solution with 1.205 x 10^{-3} mol L^{-1} $AgNO_3$ standard solution.

Table 2. Calculation of the end point by the Gran/second derivative method. Titration of 60.0 μL of a NaCl solution with a 1.00 x 10^{-3} mol L^{-1} $AgNO_3$ standard solution (average ± standard deviation of 10 replicates)

Strategy	Aliquots x Volume (μL)	Gran/2nd derivative (x 10^{-3} mol L^{-1})	rsd (%)	Error (%)
1	3 x 10	1.177 ± 0.032	2.7	+ 1.3
2	4 x 7.3	1.170 ± 0.004	3.1	+ 0.6
3	3 x 15	1.183 ± 0.018	1.6	+ 2.0

Manual method: (1.163 ± 0.007) x 10^{-3} mol Cl^- L^{-1}.

procedure was made by adding different titrant aliquots from 90% to 110% of the end point volume. Table 3 summarises the results obtained in these studies, indicating that aliquots from 1.3 to 2.3 μL provide the best precision and accuracy. Titrant increments of 4.2 μL, in spite of providing rapid titration, impair both the precision and the accuracy, as the aliquots are too large. When titrant increments of 0.9 μL were employed, the precision and accuracy of the results were also worsen because the precision of the syringe is ± 0.5 μL. In addition, this approach is time-consuming as the number of aliquots added is increased.

Table 3. Effect of the aliquot volume on the precision and accuracy in the determination of the end point by the second derivative method. Titration of 60.0 μL of a NaCl solution with a 1.00 x 10^{-3} mol L^{-1} $AgNO_3$ standard solution (average ± standard deviation of 10 replicates)

Aliquot volume (μL)	Number of points	Chloride conc. (x 10^{-3} mol/L)	rsd (%)	Error (%)
4.2	3	1.001 ± 0.052	5.2	- 1.6
2.3	5	1.026 ± 0.024	2.4	+ 0.7
1.7	8	1.014 ± 0.019	1.9	- 0.5
1.3	10	1.028 ± 0.022	2.1	+ 1.0
0.9	15	1.004 ± 0.040	4.0	- 1.5

Manual method: (1.019 ± 0.011) x 10^{-3} mol Cl^- L^{-1}.

Considering the results obtained in the above mentioned studies, the determination of chloride ions in parenteral solutions and electrolytic beverages was carried out, employing 3 additions of 15.0 μL of titrant for Gran calculation, with further end point determination by the second derivative method, by adding five titrant aliquots of 2.3 μL from 90% to 110% of the estimated end point. Table 4 lists the results obtained, indicating that, on average, the second derivative method provides better precision and accuracy than the Gran method. However, both methods show good agreement, at a confidence level of 95%, with the results obtained by manual titration, which were considered as reference values. Finally, a titration can be carried out in about 5 minutes by using the Gran / second derivative method. This time interval can be reduced to 1 minute when only the Gran method is used to find the end point.

Conclusions

The proposed system can perform a precipitating titration by employing a single aliquot of sample, consuming as low as 60 μL of sample and/or titrant solutions and providing good precision and accuracy. The fact that a true titration can be made according to the IUPAC

Table 4. Determination of chloride in parenteral solutions (PS) and electrolytic beverages (EB) with a 1.00 x 10^{-3} mol L^{-1} $AgNO_3$ standard solution (average ± standard deviation of 10 replicates)

Sample	Manual Chloride (x 10^{-3} mol L^{-1})	Gran Chloride (x 10^{-3} mol L^{-1})	Gran rsd (%)	Gran Error (%)	Gran / 2nd derivative Chloride (x 10^{-3} mol L^{-1})	Gran / 2nd derivative rsd (%)	Gran / 2nd derivative Error (%)
PS01	1.608 ± 0.003	1.595 ± 0.035	2.2	- 0.8	1.581 ± 0.027	1.7	- 1.6
PS02	1.538 ± 0.009	1.487 ± 0.045	3.1	- 3.3	1.495 ± 0.018	1.2	- 2.8
PS03	1.552 ± 0.013	1.536 ± 0.041	2.7	- 1.1	1.541 ± 0.029	1.9	- 0.8
PS04	1.075 ± 0.011	1.069 ± 0.024	2.3	- 0.6	1.083 ± 0.017	1.6	+ 0.5
PS05	0.957 ± 0.010	0.946 ± 0.013	1.3	- 1.1	0.940 ± 0.011	1.2	- 1.8
EB01	1.185 ± 0.004	1.163 ± 0.019	1.6	- 1.8	1.181 ± 0.013	1.1	- 0.3
EB02	1.349 ± 0.004	1.356 ± 0.038	2.8	+ 0.5	1.371 ± 0.023	2.0	+ 1.7
EB03	0.996 ± 0.005	1.012 ± 0.023	2.2	+ 1.5	0.997 ± 0.027	2.7	+ 0.1
EB04	1.192 ± 0.007	1.180 ± 0.032	2.7	- 1.0	1.182 ± 0.026	2.2	- 0.8

definition makes the system unique in the field of flow methodologies. In addition, the capabilities of the system can be easily expanded in order to obtain the whole titration curve, as obtained in manual methods. The time for sample processing can be chosen as a compromise between speed and accuracy by employing only the Gran method (1 min) or the Gran method followed by the second derivative method (5 min). The requirements of a given application, as a compromise between precision and time of titration, will define the choice.

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