AUTOMATED RADIOACTIVE SAMPLE PROCESSOR, THE RSP-β400

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ABSTRACT

The Radioactive Sample Processor, RSP-β400, has been designed to substantially reduce the three aspects (time, cost and effort) of sample handling and counting of beta-emitting radioisotopes using the technique of liquid scintillation counting. This is accomplished through a combination of two previously available technologies. First, a sample processor is utilized for the accurate removal of a specific variable (0.1-1.0 ml) sample volume from a disposable sample tray and the subsequent mixing of this sample with a liquid scintillation solution (4.0 ml total volume). Second, a dual counting chamber (double sample throughput) is incorporated for the accurate quantitation of the radioactivity in two samples simultaneously. The reliability, reproducibility, linearity of detector response, data handling capacities and various counting parameters are discussed in regard to the performance of the RSP-β400 instrument.

Several applications for the utilization of the RSP-β400 Sample Processor in both the basic research and clinical research areas are explored.

INTRODUCTION

The technique of liquid scintillation counting[1-4] has been used for over 30 years for the quantitation of all beta- and low-energy gamma emitting radionuclides. Twelve basic steps are involved in the prepartion, counting and disposal of samples quantitated using liquid scintillation methodology:

1. Accurate pipetting of experimental samples into scintillation vials
2. Pipetting of scintillation solution into vials
3. Labeling and capping vials
4. Shaking vials for thorough mixing

5. Placing vials in trays/box and transporting to liquid scintillation counter (LSC)
6. Loading samples into LSC
7. Setting counting parameters
8. Waiting for results
9. Removing results
10. Unloading vials from LSC
11. Storing samples or disposing radioactive waste
12. Separating into solid and liquid radioactive waste containers

In the past 15 to 20 years, various attempts have been introduced to reduce the time, effort and cost of these twelve steps. These include the following sample handling equipment or modifications of the LSC.

First, the microprocessor was utilized to reduce the time and effort by providing for a multi-user capability, unattended operation and complete data reduction for the LSC. Second, miniature vials were introduced to reduce both the amount and cost of solid and liquid radioactive waste per sample counted. Third, the auto vial capper, auto sample shaker, and auto-pipetter for dispensing liquid scintillation solutions were used to reduce the time involved in each of the three sample handling steps. Fourth, the scintillation vial compactor was introduced for the rapid separation of solid and liquid radioactive waste, and thus reducing the time and effort in the radioactive waste disposal steps. All four of these advances have been designed in order to reduce either the cost, effort or time of preparing and counting samples in the LSC, however, no innovation has addressed all three aspects until the introduction of the RSP-β400 instrument. This new, innovative, radioactive sample processor-liquid scintillation counter reduces the previously described 12 steps to four basic steps:

1. Pipette (accuracy <u>not</u> required) into wells on a 7" x 7" tray. (The only sample handling step).
2. Cover tray with tri-layer sealer.
3. Set counting conditions.
4. Remove results and dispose of, or save sample trays.

EXPERIMENTAL

Basic Theory of Operation:

The RSP-β400 Sample Processor does not utilize conventional liquid scintillation counting vials. The liquid samples are placed in 2 ml wells in a 100-sample polyethylene sample tray. The tray is covered with a tri-layer material which seals the cover to the tray, provides a self-sealing layer, and acts to wipe the pipetter assembly needle between each sample process, thus, reducing sample memory. Next, the trays are transferred to the RSP-β400 Processor, and the counting position and parameters (protocols) of counting selected.

The automatic sample preparation sequence of the RSP-β400 Processor starts with the pipetter withdrawing scintillator (4 ml minus sample volume) from the scintillator reservoir. Next, the sample (0.1-1.0 ml) is accurately withdrawn. This order of sample handling assures that the sample is disposed initially, followed by the scintillator, which acts as a rinse. The sample/scintillator is transferred into a Teflon mixing chamber (Figure 2) where mixing is completed by a gentle stream of air or N_2 bubbles. Once mixing is completed, the sample is transferred by air pressure into the first of two specially designed Teflon chambers. To assure that the counting chamber is completely full, a liquid sensor (Figure 2) is employed to stop the sample in the counting chamber (A). The sample/scintillator is then counted according to the user-selected operating protocol. After counting of the first sample (A) has commenced, the pipetter picks up 4.0 ml of a special wash solution, which was designed to wash the system completely in order to minimize sample memory. The wash is dispensed into the mixing chamber, bubbled, and washed through the second cell (B) by air pressure.

Next, the pipettor draws up scintillation solution, with the second sample processed the same as the first, except it is transferred to a second counting chamber (B). Both samples are counted at the same time, thus allowing approximately double the throughput of a conventional liquid scintillation counter. At the termination (time or counts) of the count cycle of the first sample, it is transferred by air pressure to an organic radioactivity waste container (Figure 2). The entire mixing chamber, detector lines and counting chamber (A) is washed with

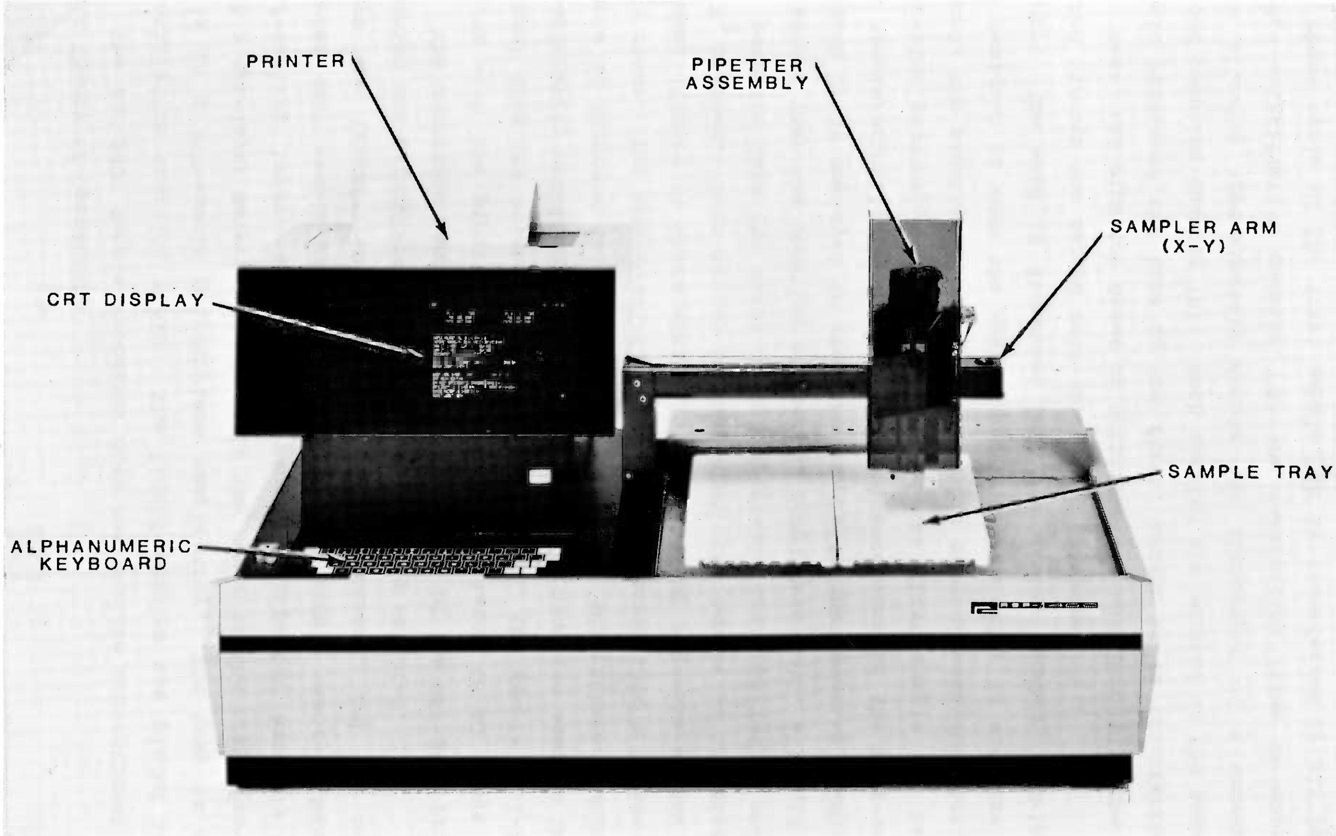

Figure 1. External View of the RSP-β400 Processor

the wash solution using the process described earlier. A third sample is then processed in the first detector (A) by the same steps described for the first sample. The process is then repeated until all of the samples in a group are completed.

RESULTS AND DISCUSSION

Because of the dual detector design and sample handling steps, the following were investigated in order to ascertain the performance of the RSP-β400 Processor:

1. Reproducibility
2. Comparison of dual counting accuracy
3. Linearity of detector/pipetter response
4. Sample memory investigation.

First, the reproducibility of sample processing was investigated by counting 100 samples (0.3 ml) each containing 5333 dpm/ml of ^{14}C. The results (Table 1) indicate that for 10-100 samples processed, the mean was 1522 cpm and the % standard deviation (SD) was 2.3. For similar samples, prepared manually, the % SD equals 2-5, therefore, the RSP-β400 instrument usually performed better than the manual method.

TABLE 1: REPRODUCIBILITY OF QUANTITATION OF RADIOACTIVE SAMPLES USING THE RSP-β400 SAMPLE PROCESSOR*

No. of Injections	Mean (CPM)	SD (CPM)	% SD
10	1500	34	2.3
20	1524	40	2.6
30	1531	36	2.3
50	1528	32	2.1
70	1529	34	2.2
90	1527	34	2.2
100	1522	36	2.3

*^{14}C glycerol tristearate dissolved in methanol, 5333 dpm/ml, with 0.20 ml sampled by the RSP-β400 Processor and counted 5.0 minutes (3 times).

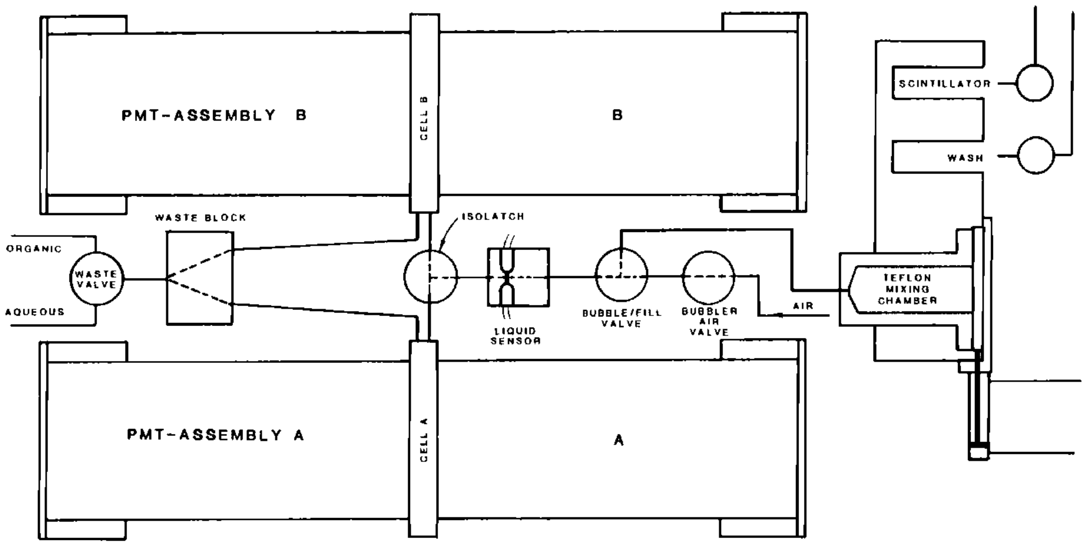

Figure 2. Diagram of Liquid Flow in the RSP-β400 System

Second, the dual detector responses of the RSP-β400 unit were investigated. the data (Table 2) demonstrates 10 samples processed in both Detector A and Detector B. The results indicating a % SD of 1-2 for the two detectors. Thus, the dual detectors can be used to count two samples simultaneously in the RSP-β400 Processor.

Third, the linearity of sample and detector response was investigated by sampling 0.1-1.0 ml of radioactive sample in the RSP-β400 Processor.

TABLE 2: COMPARISON OF QUANTITATION OF RADIOACTIVE SAMPLE USING DUAL COUNTING FEATURE OF THE RSP-β400 SAMPLE PROCESSOR*

Volume Sampled (ul)	Detector A (CPM)	Detector B (CPM)
1	1496	1496
2	1530	1587
3	1512	1525
4	1561	1549
5	1546	1530
6	1537	1510
7	1502	1558
8	1530	1549
9	1555	1584
10	1527	1502
Mean (CPM)	1518	1524
S.D. (CPM)	26	34
% S.D.	1.7%	2.2%

*(Same sample and counting conditions as used in Table 1)

The results indicate that the sampling and counting process are linear for both the sample handling (0.1-1.0 ml) and counting functions of the RSP-β400 instrument. The slope of 5.05(A), 5.08(B), and intercept of -143(A), -158(B) respectively with a coefficient of variation of 1.008(A), 1.013(B) are calculated from the above data.

Finally, with the dual detector assembly containing common liquid lines and mixing chamber, the possibility of substantial sample memory exists. The results of processing a sample containing less than 20,000 counts (18,000 cpm) results in only 0.1-0.3% memory (28 cpm) in the next

TABLE 3: LINEARITY OF SAMPLER AND DETECTOR RESPONSE OF THE RSP-β400 SAMPLE PROCESSOR*

Volume Sampled (ul)	Detector A (CPM)	Detector B (CPM)
100	440	452
100	467	456
200	846	850
200	829	842
400	1790	1774
400	1816	1781
600	2881	2821
600	2818	2762
800	3839	3874
800	3894	3750
1000	4978	5011
1000	4947	4928

*(Same sample and counting conditions used in Table 1)

sample. With one million counts processed, less than 50 cpm is retained as memory in the subsequent sample, this is accomplished by using a double wash cycle for all samples with count >20,000 cpm.

APPLICATIONS

Several applications for the utilization of the RSP-β400 Sample Processor have been explored. These include research in the basic sciences, as well as, the clinical area. First, the RSP-β400 unit can be utilized directly collecting fractions from the HPLC or low pressure column system with subsequent automatic analysis. Second, the assay of steroid receptors[5] (estradiol, progesterone, etc.) can be done in serum using a ^{3}H-substrate. At the end of the reaction, charcoal is added to remove unbound ^{3}H-steroid. This type of assay is used routinely to study reproductive disorders and several types of cancer. In a similar manner, bound and free steroids in the serum[6] can be monitored using the same basic techniques. Third, radioimmunoassays (RIA) can be done for various types of small molecules (steroids, peptides, theapeutic drugs, etc.)[7]. With this technique, it is possible to do the entire RIA in the tray used on the RSP-β400 instrument.

CONCLUSION

The RSP-β400 Sample Processor is a radioactive sample processor/ liquid scintillation counter which can reproducibility sample, process and count (dual chamber) two samples simultaneously. The two detector assemblies can be adjusted (equalized) such that both count with the same efficiency. The RSP-β400 unit is designed to have minimal sample memory.

The benefits of utilizing the RSP-β400 Sample Processor are: 89% reduction in sample handling; 62% savings in labor cost; 30-70% savings in disposables, 30-60% savings in radioactive waste disposal costs.

Therefore, the RSP-β400 instrument is very cost-effective for a scientist who requires a liquid scintillation counter (beta- or low-energy gamma-emitter) which has a high throughput of samples and requires concominant reduction in sample handling steps and cost.

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