# Optimization of a portable NIR device for the optical supervision of milk coagulation process

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## ABSTRACT

The coagulation of milk is the fundamental process in cheese-making, which is based on a gel formation as consequence of physicochemical changes taking place in the casein micelles. Monitoring the whole process of milk curd formation is a dedicated process for dairy researchers and cheese companies. In addition to advances in composition-based applications by means of NIR spectroscopy, researchers are pursuing dynamic applications that show promise especially with regard to tracking a sample *in situ* during processing The objective of this work is to propose an original portable NIR equipment to supervise the milk coagulation process. The experiments have been carried out on sheep and goat milk, by immersion of the probe directly in the liquid and acquiring spectrum each 1 minute during the 30 minutes of coagulation process. The increasing values of transflected light registered allow identifying, based on PCA analysis, the different kinetics that occur along the gel formation and the time to reach the optimal gel firmness to cut the curd.

*Keywords: goat; sheep; PCA; Hotelling T2; Q statistic; clotting kinetics.*

## INTRODUCTION

In cheese production clotting time is usually pre-deteminated using the proteolytic enzyme titulation test [1]. Thus an expert operator decides the optimum cutting time (always delayed compared to clotting time) based on his own subjective evaluation of textural and visual properties of the curd. Expert judgment usually gives acceptable results, yet variability in such optimal assessment of cutting time leads to further affects on cheese processing operations (pressing and ripening). The aforementioned reasons suggest the importance of an objective and non-destructive method to determinate optimal cutting time, which would allow indeed the automatic supervision of the cheese clotting process.

A very wide range of physical and chemical methods have been used as related to the great economic importance of this gelation process in cheese production. Many of these are non-destructive though providing only limited information on the rheological properties of the gel, and thus only few of such techniques appear attractive as possible in-vat coagulation sensors. On line, near infrared reflectance (NIR) systems are already commonly used to standardize the composition of cheesemilk in large factories so monitoring the coagulation process seems an obvious next step in gaining more control over the cheesemaking process [2]. In addition to advances in composition-based applications of NIR spectroscopy, researchers are pursuing dynamic applications that show to be promising, especially with regard to tracking a sample *in situ* during processing [3]. In this way a diffuse fiber optic near infrared (800 nm) reflectance sensor was successfully tested by Castillo et. al [4-6] to predict cutting and clotting time of milk. Using the CoAguLite sensor these authors monitored increasing diffuse reflectance during the coagulation process of goat´s milk, since turbidity and casein micelles size are affected by the process. Recently a novel online sensor technology for continuous monitoring of milk coagulation was tested by Fagan et al. [7, 8]. Their prototype used an optical probe directly installed in the vat wall which is composed, among other elements, by a tungsten halogen light source (spectral range of 360–2000 nm) and a glass window to the sample with a large field of view (LFV) relative to curd particle size. The large-diameter glass window allows scattered light to be collected from a large area, light is transmitted through a 800 μm diameter fiber optic cable to the master unit of a dual miniature fiber optic spectrometer. The LFV sensor was sensitive to both aggregation of casein micelles and development of curd firmness.

NIR spectroscopy is a rapid, non-destructive, and non-polluting technology that usually is associated to a costfull and delicate instrumentation, but since ten years ago, the development of new and more robust

spectrophotometer equipments, smaller and versatile enough, with low acquisition cost, make it possible to build portable and affordable complete NIR spectroscopy equipments. **The objective of this work is to propose an original portable NIR equipment to supervise the milk coagulation process.**

## MATERIALS & METHODS

Unpasteurized and unhomogenized milk was obtained directly from local dairy farms. Milk was pasteurized at 75ºC for 15s using a thermostatic water bath, while milk temperature was monitored using a precision thermometer (model HH2001TC, Omega Engineering Inc.). After pasteurization, milk was quickly cooled to 4ºC and stored in the cold room overnight, following the flow chart of experimental design shown in Figure 1.

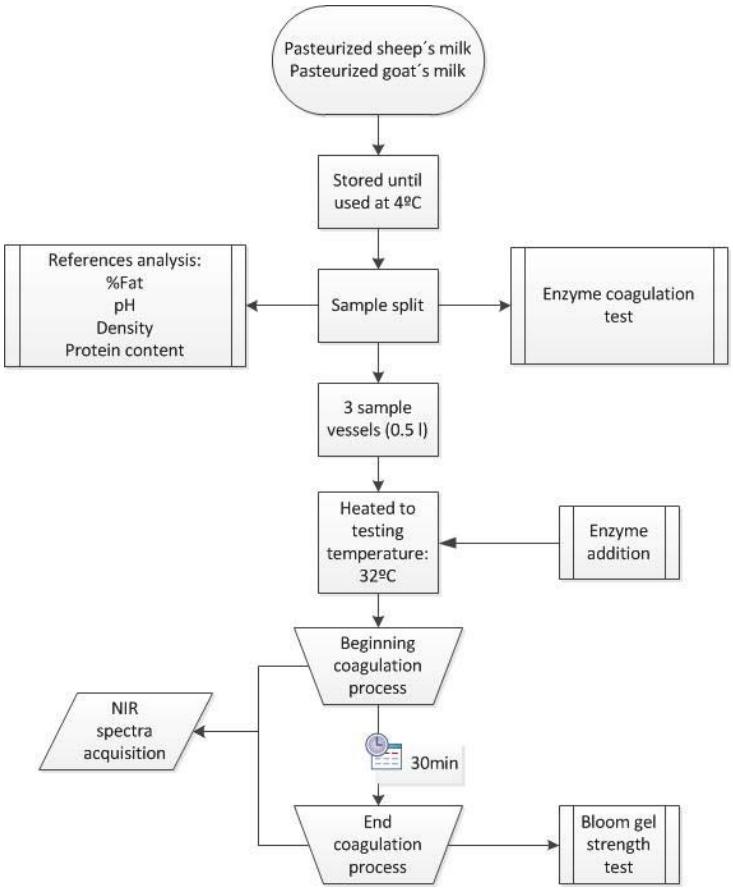



**Figure 1**. Flow chart of experimental design.

On the day of experimentation several samples of milk were taken to determine some parameters for milk characterization. The values of pH (CRISON pH Meter, GLP21), fat percentage (standard Gerber method), density (kg/l with lactodensitometer) and protein (g/l by formol titration method) for sheep´s and goat´s milk are shown in Table 1. A parallel sample was used under enzyme coagulation test, allowing to compute the concentration of coagulating enzyme (chymosin, Granday ® 6000) that leads to clotting times of 30min.

To obtain the curd, 500ml of milk were placed inside a glass vessel (600 ml of volume) and then slowly heated to the coagulation temperature in a thermostatic bath, while milk temperature supervision was carried out using a precision thermometer. The chymosin enzyme was added to the pre-heated milk at 32ºC and stirred for 1min. Once coagulating enzyme has been added the acquisition of NIR spectra started.

**Table 1.** Pasteurization treatment and some properties of whole milk used in this work

| Experiment number | Origin | Repetition | Pasteurization treatment T (ºC) + time (s) | Concentration of coagulating enzyme (ppm) | Density (kg/l) | Fat (%) | Protein (g/l) | pH |
|---|---|---|---|---|---|---|---|---|
| 1 | Sheep | 1 | 72 + 15 | 280 | 1.031 | 7.73 | 5.33 ± 0.27 | 6.68 |
| 2 | Sheep | 2 | 72 + 15 | 280 | 1.031 | 7.73 | 5.33 ± 0.27 | 6.68 |
| 3 | Sheep | 3 | 72 + 15 | 280 | 1.031 | 7.73 | 5.33 ± 0.27 | 6.68 |
| 4 | Goat | 1 | 72 + 15 | 180 | 1.030 | 5.3 | 3.38 ± 0.17 | 6.62 |
| 5 | Goat | 2 | 72 + 15 | 180 | 1.030 | 5.3 | 3.38 ± 0.17 | 6.62 |
| 6 | Goat | 3 | 72 + 15 | 180 | 1.030 | 5.3 | 3.38 ± 0.17 | 6.62 |

Laboratory NIR tests were conducted with a portable equipment (see details in Figure 2) developed by the Physical Properties Laboratory and Advanced Technologies in Agrofood of the Technical University of

Madrid. A Cooled NIR I of Hamamatsu was selected, which has a Peltier and an electric fan. This device works within a wavelength's range between 800nm and 1700nm, and can be plugged to a computer by a USB cable. A continuous light source is used which varies between 360nm and 2000nm, model LS-1-LL Tungsten Halogen, of Ocean Optics. It can provide light up to 10000 hours while its weight and its size are very little. The transmission of the light from the source through the sample torward the spectrometer is made by means of an optical fiber's guide which is diverged T200-RT-VIS-NIR, and SMA 905 connectors are used. This transflectance probe is especially useful for embedding into process streams for in situ, real-time monitoring. In transflectance operation, light is transmitted from the illumination fiber through a plano-convex lens and through the sample compartment to a flat, second-surface mirror. The light reflects form this mirror and is focused by the lens onto the read fiber, the main advantage is the reading amplification effect, measuring both transmitted light and backscattered light from the sample.
The spectra were acquired exactly at wavelength ranging from 878.6nm to 1714.7nm at 1.6 nm intervals, leading to 516 data points. The 16 bit A/D converter of the spectrometer leads to $2^{16}$ (65536) intensity counts as input light intensity. Continuous monitoring of clotting process was achieved by acquiring 10 spectra each 1min along a clotting time of 30 min (350 spectra by sample). Samples were randomly replicated 3 times with a total of 6 samples tested. Data collection was camed using "Interfaz Gráfico" software (own development on LabView), and data analysis was carried out using the Statistic Toolbox of MATLAB to perform statistical analysis. Principal component analysis (PCA) was used to investigate the raw spectra data, computing the process control statistics (Hotelling T2 and Q).

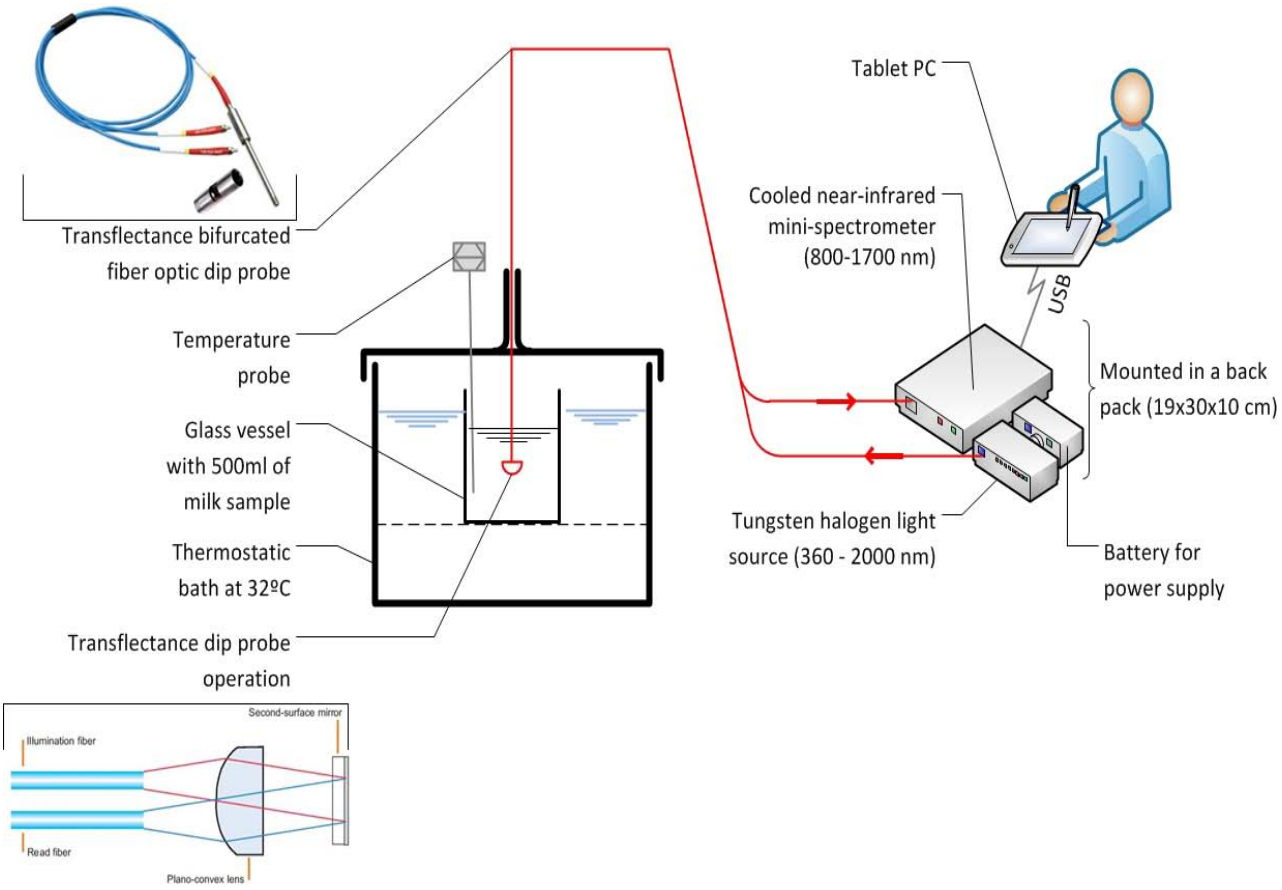



**Figure 2**. Scheme of NIR test procedure

## RESULTS & DISCUSSION

The 3D data representation using a surface graphic (see **Figure 3**) a quick verification of coagulation, since increasing light intensity is registered by the spectrometer detector. Previous authors [4] reported light backscatter increases 92% for cottage cheese, 30% to 50% for enzymatic coagulation of cow milk and

between 15% and 21% for enzymatic coagulation of goat milk. In this study, it is reached a mean intensity signal increase, for wavelength between 1200nm and 1300 nm, of 28.6 % for sheep milk and of 14% for goat milk.

In this work PCA analysis is used to compress spectral information to a few linear combinations of the original data. The new smaller set of variables, called principal components (PC), quantifies the subtle changes of the spectra from one coagulation time to another. Thus it is possible to describe a large proportion of the variability in the data with a much smaller number of variables [9]. **Figure 5** shows the biplot graph of PC1 versus PC2 from the PCA analysis carried out on sheep milk coagulation process. The first PC is defined mainly by wavelengths between 1200-1300 nm and represents the 99.987% of explained variance. While PC3 with only 25 ppm of explained variance seems to be very sensitive to coagulation process, grouping the information corresponding mainly to wavelengths between 1400nm and 1500nm (see **Figure 4**). The identification on **Figure 5** of the three important stages in the kinetics of rennet-induced coagulation of milk [10]: ❶ destabilization of the colloidal milk system by the enzymatic hydrolysis of k-casein; ❷ aggregation of the para-k-casein micelles, observed as milk flocculation and ❸ formation and development of a cross-linked gel, initiating the coagulation, allows us to establish a relationship between coagulation phases, PCs and wavelengths.

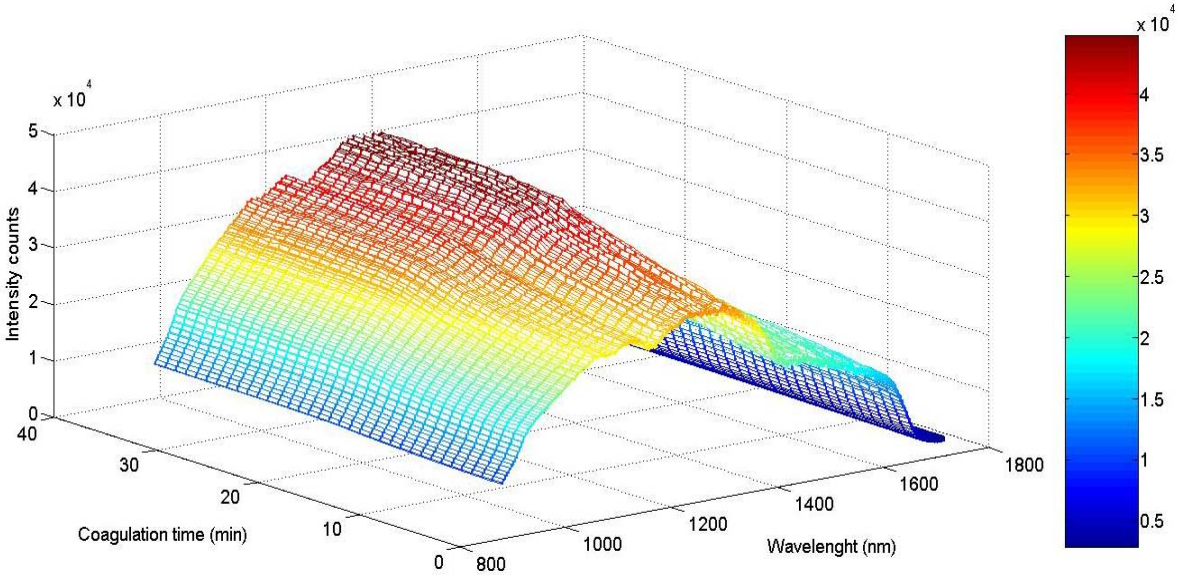



**Figure 3.** Surface graphic for light intensity recovered for each wavelength and coagulation time. Color bar represents the intensity counts values.

PC1 is especially sensitive to the aggregation phase. In this phase is reported a decrease in the particle number from 550 particles per 1$\mu m^2$ during the first 15 min to 120 particles/$\mu m^2$ at about 20 min (results for reconstituted skim milk [10] ) For the case represented in **Figure 5**, the aggregation phase goes from 12min to 19 min, and this increase in particle size is the responsible for the inflexion point that occurs in the surface graph (**Figure 3**) around this 12 min, corresponding to wavelengths between 1200-1300 nm. Lagaude et al [10] described that before and after the aggregation phase the particle number remains more or less constant, being the change in the particle number during the aggregation phase the most patent process that occurs during milk coagulation. On the other hand, PC3 follows the evolution of the gelation process during the destabilization phase (0min-12min) leading to increasing intensity values for 1400nm-1500nm wavelengths, and especially during the gel development phase (19-30 min), where it is possible to identify the decreasing gap when the 30 min of clotting process is reached.

From the point of view of the process control statistics, which refers to the Hotelling´s T2 has been computed, as a multivariate statistic which may be used as an event indicator, statistical measure of the multivariate distance of each observation from the center of the data set. Besides the Q statistic, is also computed to address atypical observations as an indicator of how well each spectrum fits the PC model [11].

In **Figure 6** the positioning of samples corresponding to 0 min and 30 min of coagulation time, how to be above the T2 limit, which refers to two events, the beginning and the end of the coagulation process. Once

the gel is completely formed and has acquired enough hardness, being more similar to a solid than a liquid, the PC model is not able to well represent spectra corresponding to these samples, and therefore in **Figure 6**, samples above 30 minutes of coagulation time, become out of Q limit. It is important to state that even though the process was follow as to reach 30 min coagulation time, the exact ending varied 10% depending on samples.

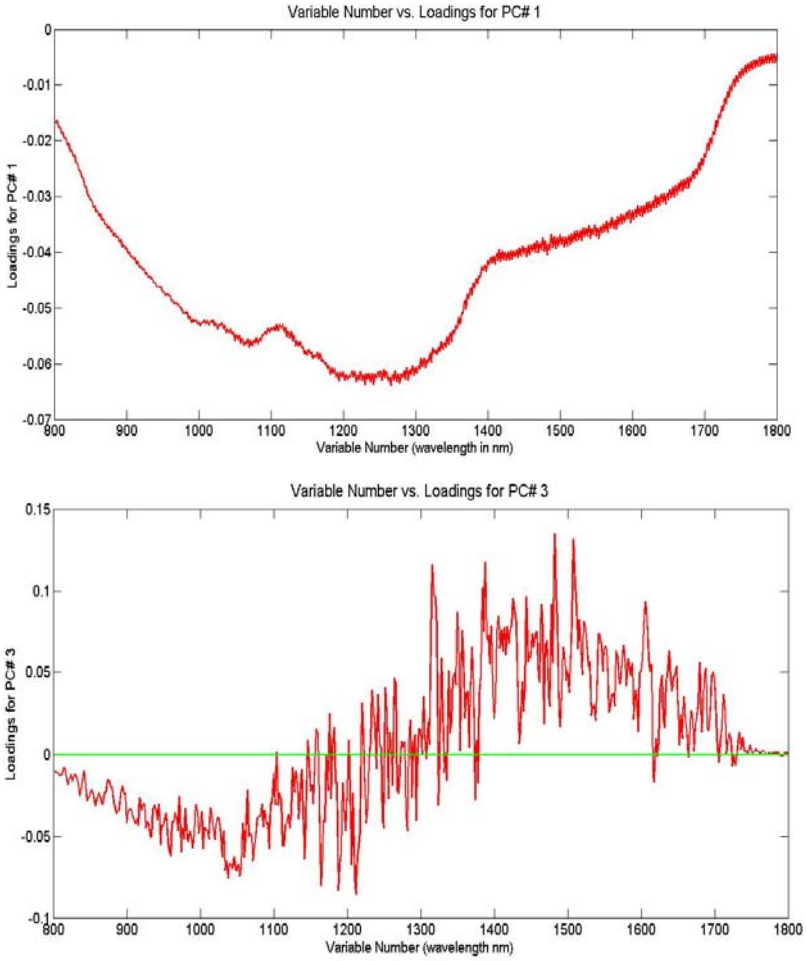
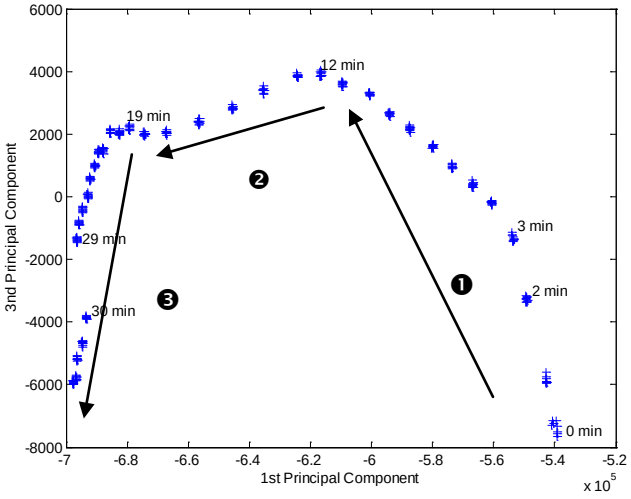



**Figure 4.** Loadings plots of the PCA model developed for sheep milk coagulation process, for the first (up) and third (down) principal component.

## CONCLUSION

The application of world wide accepted multivariate techniques like PCA combined with process control statistics (Hotelling T2) enables events to be identified in the coagulation process, while the use of the Q statistic commonly used for process control may easily be implemented in real time analysis for addressing spectra which are not well reproduced within the PC space. In this study, the final point of coagulation process can be identified as a “strange event”. “Strange” because it is not well reproduced within the PC space and “event” because it is abrupt. This final point of coagulation reached around 30 min (see green circle at **Figure 6**) is characterized by its value being out of limit for Hotelling T2 and Q statistics, and is identified as the optimum cutting time (similar results has been found in goat milk coagulation process).
Another important result or outcome of this work is the interest of studying the evolution in time of the PC scores of individuals, where each inflexion point marks the particular change towards a different coagulation process kinetics, following the spectral change in the sample minute to minute, and identifying the wavelength that enable the assessment of this coagulation process. Wavelengths between 1400nm and 1500nm refer the gel development phase and enable to identify the final gelation point as the optimum cutting time.




❶ Destabilization phase
❷ Aggregation phase
❸ Gel development phase

**Figure 5.** Biplot of scores on principal components 1 and 3 of samples corresponding to sheep milk.

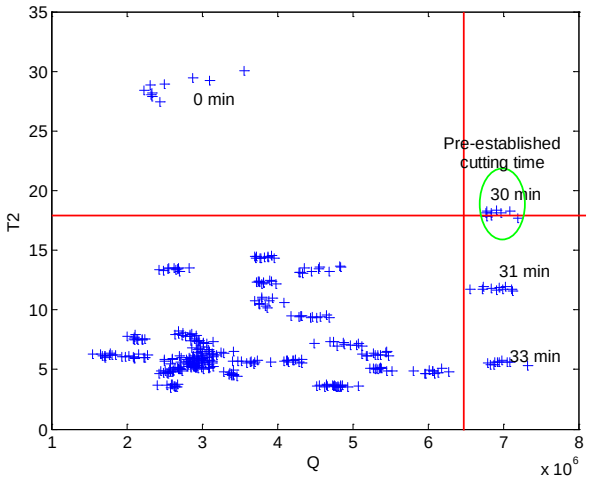



**Figure 6.** Biplot of Q vs T2 statistics of the PCA model developed for sheep milk coagulation process. Red lines indicate the T2 limit (horizontal) and Q limit (vertical).


## ACKNOWLEDGEMENTS

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